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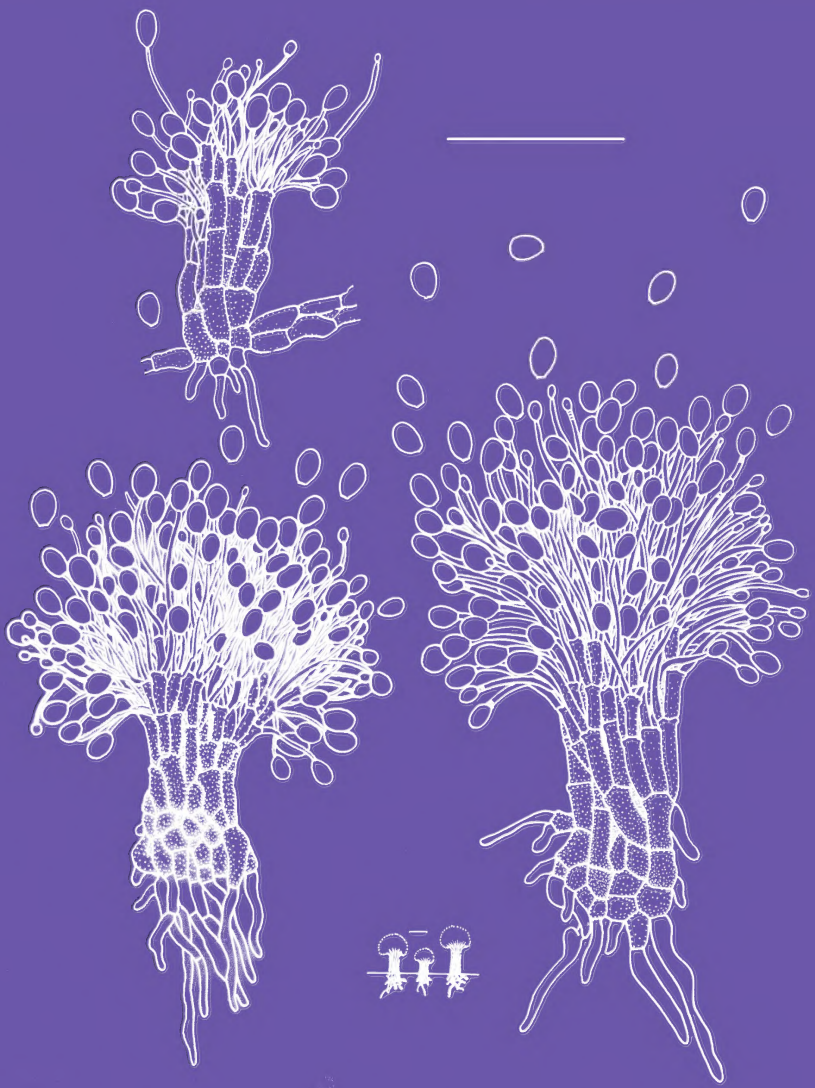

Graphium wuweiense sp. nov.
(Wu, Xu, & al. — FIG. 2, p. 399)
YUE-MING WU, *artist*

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p. 44, line 10	for: *Sporidesmium*-like genera, are polyphyletic (Shenoy read: *Sporidesmium*-like genera, is polyphyletic (Shenoy
p. 44, line 34	for: and sometimes the conidium between the read: and sometimes the portion of the conidium between the
p. 54, line Table 2	for: Dai et al. 2007 read: Dai YC, Yu CJ, Wang HC (2007) Polypores from eastern Xizang (Tibet), western China. Ann Bot Fennici 44: 135–145.

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Reviewers — volume one hundred twenty-nine (two)

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From the Editor-in-Chief

A fond au revoir to Else — Responding to a series of wheedling editorial Emails during late 2010, the esteemed Dr. Vellinga agreed to assume the mantle of Book Review Editor from David Hawksworth—but with only (alas) a one to two year commitment. Her fourth year now concluding and with the California drought finally promising to break, Else wishes to relinquish her post to make time for intensive field and lab research. We are sad to lose her but hope to publish some of her research (and perhaps an occasional book review?) in the not too distant future.

Mycotaxon 129(2) contains 30 research papers by 115 authors (representing 23 countries) that have been previously revised by 115 expert reviewers.

Within its pages are two new genera (*Rhizoglomus* for arbuscular mycorrhizal fungi previously placed in *Rhizophagus* and *Sedelnikovaea* for a distant relative of *Protoparmeliopsis*), one new subgenus (*Postia* subg. *Cyanosporus*), and 28 species new to science representing *Coccomyces, Cytospora, Dictydiaethalium, Diploschistes, Graphium, Leptostroma, Myrothecium, Periconia, Podosphaera, Pseudocercospora, Psilocybe,* and *Uncispora* from China; *Entoloma* from Australia; *Galerella* from Mexico; *Laboulbenia* from The Philippines; *Lactarius* and *Leptocorticium* from India; and *Stemonitis* from Turkey.

In addition to range extensions and/or new hosts for previously named taxa, we also offer 18 new combinations in *Postia, Rhizoglomus,* and *Sedelnikovaea,* a new typification for *Umbilicaria kisovana,* an examination of the potential of *Neozygites* for aphid pest control in Chile, and an excellent molecular analysis of Murrill's southeastern U.S.A. *Russula* type specimens.

Onward to 2015 — Mycotaxon is pleased to close 2014 having successfully published a total of five issues (1267 pages), 140 research papers, and 144 new taxa. Editorial health has improved and is holding, making attainable our new year's goal of publishing each quarterly well within the dates emblazoned on its spine (instead of far too many weeks afterwards). We take this opportunity to wish all our readers, authors, and reviewers the happiest, healthiest, and most productive New Year.

Warm regards,

Lorelei L. Norvell (*Editor-in-Chief*)
30 December 2014

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Morphological and molecular evidence for a new species of *Psilocybe* from southern China

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Abstract—*Psilocybe cinnamomea* sp. nov. is reported from China. It is characterized by its campanulate to plano-convex pileus, ellipsoid spores with a distinctly broad germ pore, clavate basidia, polymorphic cheilocystidia, and clavate to fusiform pleurocystidia with a short unbranched neck. Phylogenetic relationships among the new species and allied species in the genus were inferred from the internal transcribed spacer (ITS) region.

Key words—*Agaricales, Strophariaceae,* taxonomy, phylogeny

Introduction

Psilocybe (Fr.) P. Kumm. was formerly a broadly circumscribed genus, including bluing, hallucinogenic species and non-bluing, non-hallucinogenic species (Guzmán 1983, Allen et al. 1992, Stamets 1996, Guzmán 2005, Borovička et al. 2011, Guzmán 2012). Recently, this broad polyphyletic genus was divided: *Psilocybe* sensu stricto (with *P. semilanceata* as its conserved type) was restricted to the bluing species, while the non-bluing species were assigned to *Deconica* (W.G. Sm.) P. Karst. (Redhead et al. 2007, Norvell 2010).

Psilocybe s. s. contains more than 150 species worldwide (Guzmán 2005, 2009). However, only 11 species have been reported from China, of which only three were originally described from the country (Bau & Sarentoya 2009, Guzmán & Zhu L. Yang 2010, Ma et al. 2014). In the present study, a new species of *Psilocybe* is described from southern China. In order to confirm the phylogenetic uniqueness of this species, sequences of its internal transcribed spacer (ITS) were generated and compared with sequences of other *Psilocybe* species.

Material & methods

Field collections were conducted in 2010. Notes and photographs were taken on macro-morphological features, and specimens were dried using a Dörrex dehydrator at 50°C. Specimens were deposited in the Research Institute of Tropical Forestry (RITF),

Chinese Academy of Forestry. Macroscopic descriptions were based on the photos and notes made in the field. Descriptive terminology follows Vellinga (1988), and colours are designated according to Kornerup & Wanscher (1981).

For microscopic observations, sections of specimens were cut by hand and mounted in 5% aqueous KOH solution or 1% aqueous Congo red solution. Basidia, basidiospores, cheilocystidia, pleurocystidia, caulocystidia, and pileocystidia were measured using the MShot Digital Imaging System (n=25 for each character). The abbreviation [n/m/p] indicates that measurements were made on n basidiospores in m basidiomata from p collections. Basidiospore dimensions follow the form (a–)b–c(–d) with b–c representing 95% of the measured values and extreme values shown in parentheses.

DNA was extracted from herbarium materials (Zhou & Liang 2011). The internal transcribed spacer (ITS) regions were amplified by polymerase chain reaction (PCR) with the primers ITS1 and ITS4 (White et al. 1990). Both strands were sequenced with an ABI 3730 DNA analyzer and an ABI BigDye 3.1 terminator cycle sequencing kit (Beijing AuGCT DNA-SYN Biotechnology Co., Ltd, Beijing). The newly generated sequences were submitted to GenBank (accession numbers KJ433483 and KJ433484). DNA sequences were edited and aligned using SeqMan (DNA STAR Package) and Clustal X (Thompson et al. 1997) and manually checked and adjusted. Ambiguous positions were excluded from the matrix. The dataset was analyzed with the RAxML BlackBox online server (Stamatakis et al. 2008) for the maximum likelihood and MrBayes 3.1 for bayesian inference (Huelsenbeck & Ronquist 2005).

Taxonomy

Psilocybe cinnamomea J.F. Liang, Yang K. Li & Ye Yuan, **sp. nov.** Fig. 1

MycoBank 807941

Differs from *Psilocybe zapotecorum* by its smaller basidiomata, its greenish white to pale green lamellae, and its smaller basidia.

Type: China, Guangdong Province, Lechang County, Yangdongshan Shierdushui Nature Reserve, 4 October 2010, Wang 189 (**Holotype**, RITF 746; GenBank KJ433484).

Etymology: The Latin word "*cinnamomea*" refers to the cinnamon-like color of young basidiomata.

Basidiomata small. Pileus 20–40 mm in diameter, campanulate when young, expanding to subconvex or plano-convex, slightly papillate at the apex, glabrous, hygrophanous, cinnamon (5B3–5C6) when young, surface pale (2A2) to cream (4A2–4), light orange (5A4–5B7) at the disk when mature, yellowish brown (5C4–8) in dry specimens, blackish blue with age or when bruising, margin with white floccose remnants from the veil. Lamellae subadnate or sinuate, crowded, unequal, greenish white to pale green (28A2–3). Stipe 20–80 × 2–5 mm, uniform or slightly thickened at the base, somewhat flexuous, hollow, whitish to pale green (5B3) at the upper, grayish orange (5B3–5) to brownish yellow (5C6–8) at the lower part, covered with milk white (1A2) fibrillose, bluing, blackish when dried. Context thin, whitish. Odor or Taste not recorded. Annulus absent.

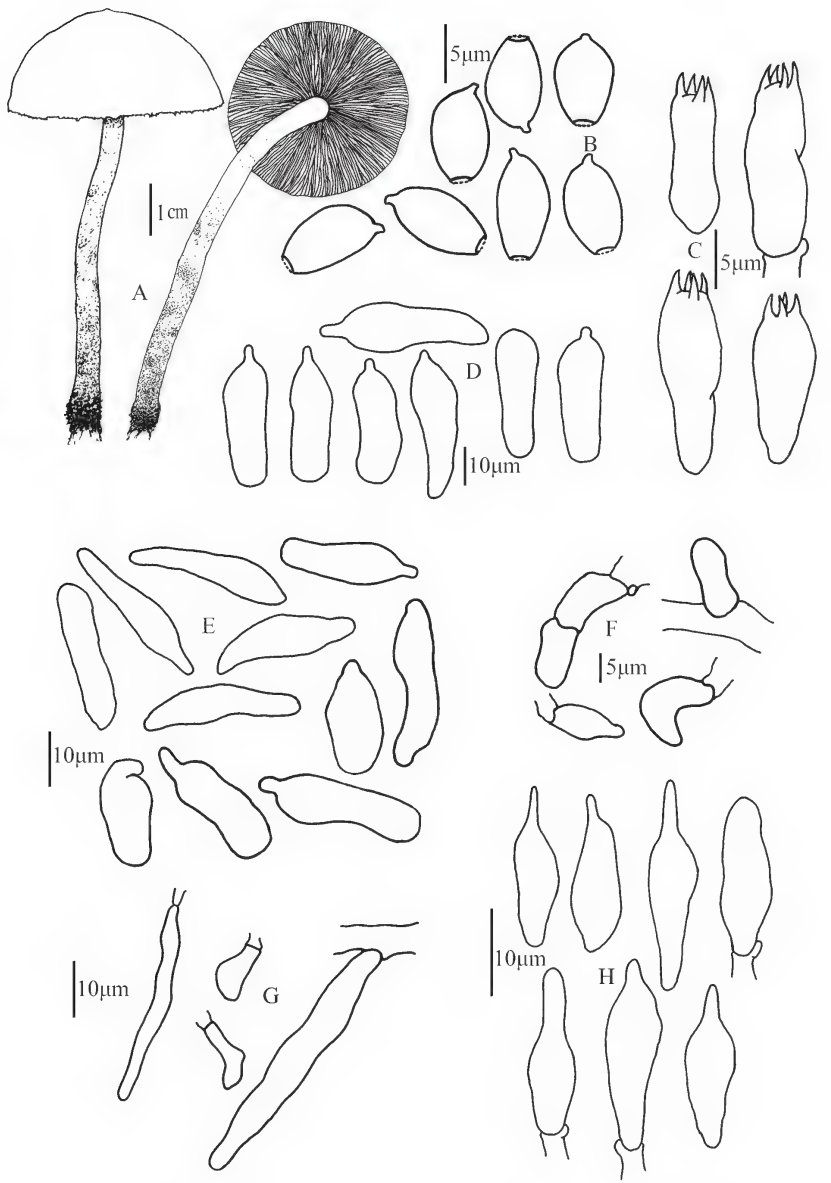

Fig. 1 *Psilocybe cinnamomea.* A. Basidiomata. B. Basidiospores. C. Basidia. D&H. Cheilocystidia. E. Pleurocystidia. F. Pileipellis. G. Caulocystidia. (A–G from holotype, RITF 746; H from RITF 726).

Basidiospores (5.5–)6.5–7.5(–8) × (3–)4–4.5(–5) μm, ellipsoid to subovoid in face view, subellipsoid in side view, sometimes subventricose, smooth, thin-walled, wall about 0.3–0.5 μm thick, with a distinctly broad germ pore and a short apical appendage, yellowish-brown pigment in KOH. Basidia 7–13.5 × 3.0–6.0 μm, 4-spored, rarely 2-spored, hyaline, clavate, sometimes with a median constriction. Cheilocystidia common, (17.5–)20–30 × 6.5–10 μm, unbranched, polymorphic (subcylindrical, clavate-mucronate, utriform, lageniform, fusiform), frequently with a median constriction and with a short or long neck, hyaline in 5% KOH. Pleurocystidia abundant, 17.5–32 × 5.5–10.0 μm, clavate, narrowly fusiform to fusiform, frequently with a short neck, the neck ≤5 μm long, unbranched, hyaline, walls smooth. Pileipellis a subcutis, not subgelatinized, 9–13 μm thick, hyaline, hyphae 3–5 μm diam., rarely with surface subcylindric or fusiform cystidioid elements 7.5–17.5 × 4.5–7.5 μm. Caulocystidia rare, 9.5–85 × 5–11 μm, variable in shape (subcylindrical or attenuate). Clamp connections common.

Additional specimen examined: **CHINA**: Guangdong Province, Lechang County, Yangdongshan Shierdushui Nature Reserve, 4 October 2010, Wang 234 (RITF 726; GenBank KJ433483).

Habitat & Distribution – Gregarious to caespitose on rotten wood or nutrient rich soils in a subtropical evergreen broad-leaved forest. Known only from the type locality.

Phylogenetic analyses

A dataset of ITS sequences with 700 nucleotide sites was analyzed for 42 taxa (including 41 from GenBank). The dataset contained available sequences of *Psilocybe* species; *Stropharia rugosoannulata* Farl. ex Murrill and *S. hornemannii* (Fr.) S. Lundell & Nannf. were chosen as outgroup.

Bayesian and RAxML phylogenetic analyses (Fig. 2) clustered together two *P. cinnamomea* sequences on a well-supported branch (bootstrap = 100%; posterior probability = 1.00) and placed *P. cinnamomea* with *P. antioquiensis* Guzmán et al., *P. zapotecoantillarum* Guzmán et al., *P. zapotecorum* R. Heim emend., *P. thaizapoteca* Guzmán et al., and *P. argentipes* K. Yokoy. in the same subclade /zapotecorum (bootstrap = 87%; posterior probability = 1.00).

Discussion

Psilocybe cinnamomea is easily recognized by its campanulate to plano-convex pileus, ellipsoid spores with a distinctly broad germ pore, clavate basidia, polymorphic cheilocystidia, and clavate to fusiform pleurocystidia with a short unbranched neck. Its ellipsoid thin-walled spores and bluing discoloration suggest placement of *P. cinnamomea* in *P.* sect. *Zapotecorum* (Guzmán 1983).

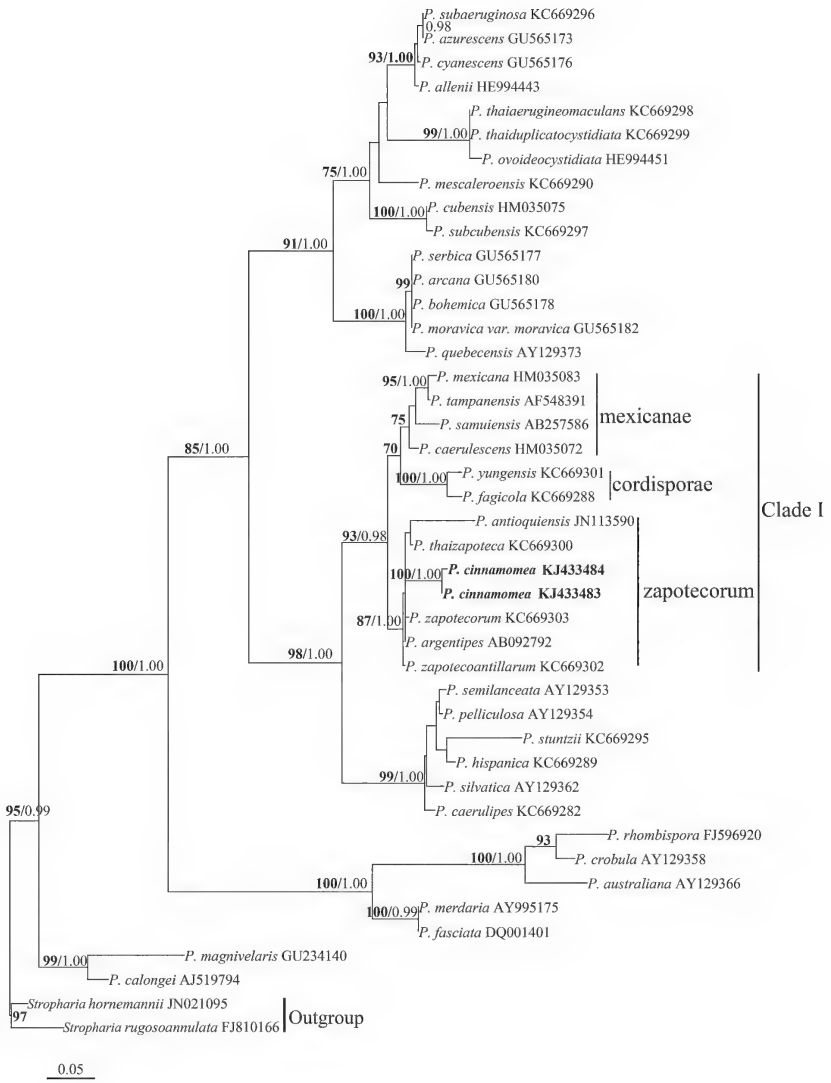

FIG. 2 One of 100 RAxML likelihood trees (–ln L 5242.090489) based on the *Psilocybe* ITS dataset. Support values in bold type are RAxML likelihood bootstrap (≥70%). Values in normal type are Bayesian posterior probabilities (≥0.95).

Observations of both collections suggest that *P. cinnamomea* shows considerable variation in shape and size of cheilocystidia. In young specimens (Fig. 1H), the cheilocystidia are utriform to lageniform (17.5–22.5 × 3.5–5.5 μm) with a long neck (≤6.5 μm long), while those in mature specimens (Fig. 1D) are subcylindrical to clavate-mucronate with a short neck. Although there are differences between young and mature specimens, they are confirmed as representing the same species based on their same habitat and an ITS sequence similarity between young and mature specimens of 99%.

Bayesian and RAxML phylogenetic analyses (Fig. 2) supported *P. cinnamomea* in Clade I, representing hallucinogenic and mainly tropical and subtropical taxa (Guzmán et al. 2012) that were divided into three subclades (/cordisporae, /mexicanae, /zapotecorum) that grouped together with 93% bootstrap support and 0.98 posterior probability. Within /zapotecorum, *P. antioquiensis*, which has slightly thick-walled and angular spores, has been placed in *P.* sect. *Mexicanae* (Guzmán et al. 1994), while the other species, which share ellipsoid, thin-walled, non-angular spores, have been assigned to *P.* sect. *Zapotecorum* (Guzmán et al. 2003, 2012).

As *P. cinnamomea* bruises or dries blackish blue, it might also be hallucinogenic.

Closely related to *P. cinnamomea* are *P. subcaerulipes* Hongo (from Japan), which differs by its distinctly subumbonate brown to orangish brown pileus, gray-brown to dark violaceous brown lamellae, longer basidia (15–25 × 4.5–6 μm), and irregularly branched pleurocystidia (Guzmán et al. 2013); *P. zapotecorum* (from Mexico), which has larger basidiomata, whitish-brown to dark violaceous lamellae, and larger basidia (Guzmán 2012); and *P. zapotecoantillarum* (from Puerto Rico), which is easily distinguished by light brown to dark reddish-brown adnexed lamellae, longer basidia, and smaller ventricose pleurocystidia (Guzmán et al. 2003).

Psilocybe antioquiensis, described from Colombia, is close to *P. cinnamomea* in phylogenetic analyses but is distinguished by its reddish brown to fulvous pileus, gray brownish or violaceous brown lamellae, bigger angular spores (6–10.6 × 4.5–7 μm), and longer basidia (20–30 × 6–8 μm; Guzmán et al. 1994).

Morphologically, the new species is similar to the Caribbean species *Psilocybe guilartensis* Guzmán et al. and the Mexican species *P. moseri* Guzmán. However, *P. guilartensis* (placed in *P.* sect. *Brunneocystidiatae*) has thick-walled rhomboid spores and longer basidia (20–32 × 4–7 μm; Guzmán et al. 2003), while *P. moseri* has a dark buff to brownish pileus, brownish lamellae, and smaller spores (4–6.5 × 3–3.5 μm) and pleurocystidia (12–17 × 4–5.5 μm; Guzmán 1995).

Acknowledgments

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Powdery mildew on *Bougainvillea spectabilis* in Mexico with a cryptic generic affinity

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Abstract —An asexual powdery mildew on *Bougainvillea spectabilis* resembles *Ovulariopsis* (anamorphic *Phyllactinia* and *Pleochaeta*), while its combination of sinuous to coiled conidiophores forming dimorphic conidia singly suggests a *Pleochaeta* anamorph. However, this severely contradicts the rDNA ITS molecular data. The *Bougainvillea* powdery mildew shows the closest genetic similarities (80-83% sequence homologies) with various *Leveillula* and *Phyllactinia* species (*Phyllactinieae*), whereas *Pleochaeta* species were not genetically close. These results suggest that the *Bougainvillea* powdery mildew has an affinity with tribe *Phyllactinieae* of the *Erysiphaceae*, but the percentage of genetic similarity is below the scope of generic limits in powdery mildew ITS data. Although a new genus may be involved, we cannot formally propose it based on a single sequence, an anamorph morphologically indistinguishable from *Ovulariopsis,* and an unknown teleomorph.

Key words— *Erysiphales*, ornamental plant, new species, Michoacan

Introduction

Bougainvillea is a widely used ornamental plant in Mexico, with Morelos state one of the main producers in the country. In Michoacan it is the most common ornamental used in gardens, avenues, and parks. The flowers are also used for medicinal purposes, mainly as remedy for respiratory illnesses. The powdery mildew *Leveillula taurica* (Lév.) G. Arnaud has been associated with *Bougainvillea* in Mexico (Ramírez-Rojas et al. 2010), but its identification

has not yet been confirmed by molecular methods. The objective of this study was to determine the identity of the pathogen causing powdery mildew on 100–250 cm tall flowering *Bougainvillea spectabilis* plants collected during July and September 2009 in two urban municipalities in Michoacan, México. Some *Bougainvillea* plants showed chlorotic lesions on both sides of the leaves. Lesions were small (3–6 mm diameter), irregularly shaped, and with a central necrotic area. Most mature leaves of the affected plants showed lesions on the upper side of the leaves but lacked mycelia and other fungal reproductive structures. Lesions on the undersides were colonized by sparingly developed mycelia with few conidiophores and conidia.

Materials & methods

Two hundred forty-six *Bougainvillea spectabilis* plants were examined for powdery mildew in Morelia and Tarimbaro, municipalities in Michoacan state. Powdery mildew colonies on infected plants were prepared by means of a dissecting microscope and examined by fresh mounts in distilled water (with or without aniline blue) or dry mounts in 85% lactic acid, with staining and gentle heating (modified from Shin & La 1995 according to technique 5 in Liberato et al. 2005) and using standard light microscopy with oil immersion (bright field and phase contrast, Olympus BX 50, Hamburg, Germany). 30 measurements (1000× magnification) of conidia and other structures were made. Voucher specimens were conserved in the herbariums at Universidad Michoacana de San Nicolás de Hidalgo, Morelia, Michoacán, Mexico (EBUM) and Martin-Luther-Universität, Halle, Germany (HAL).

Genomic DNA was obtained from powdery mildew infected leaves from samples collected in gardens. Standard protocols followed instructions of the manufacturer (Wizard Genomic DNA Purification Kit, Promega Corp., Madison, Wisconsin). The internal transcribed spacer (ITS) region of nuclear ribosomal DNA, including 5.8S rDNA, the 3' end of 18S rDNA and the 5' end of 28S rDNA, was amplified from powdery mildew according to Cunnington et al. (2003). PCR reactions were carried out using HotStarTaq DNA polymerase (HotStarTaq Master Mix Kit, Qiagen, Valencia, CA) in a thermal cycler (Mastercycler Gradient, Eppendorf). The PCR DNA products were cleaned using a commercial kit following the manufacturer's protocol (Wizard SV Gel and PCR Clean-up System (Promega, Corp., Madison, Wisconsin). PCR products were visualized by electrophoresis in 1.5% agarose gels in TAE buffer. Direct sequencing of the PCR products was carried out by an external company (Macrogen Inc. Seoul, Korea). Programs Pregap and Gap in the Staden Package (http://staden.sourceforge.net) were utilized to obtain consensus sequences for the amplified region from each isolate. Sequences were deposited in GenBank, 626 bp as *Ovulariopsis* sp. GRA-2013 (Accession no. KC556804). The sequences concerned were used in the BLASTN 2.2.25+ program (NCBI http://www.ncbi.nlm.nih.gov/) to determine the identity or at least the affinity based on sequence similarities expressed as percent sequence identity. A restricted phylogenetic tree was prepared, using the program Mega5 by neighbor-joining method with Tajima-Nei distance calculation to demonstrate relationships to the closest relatives.

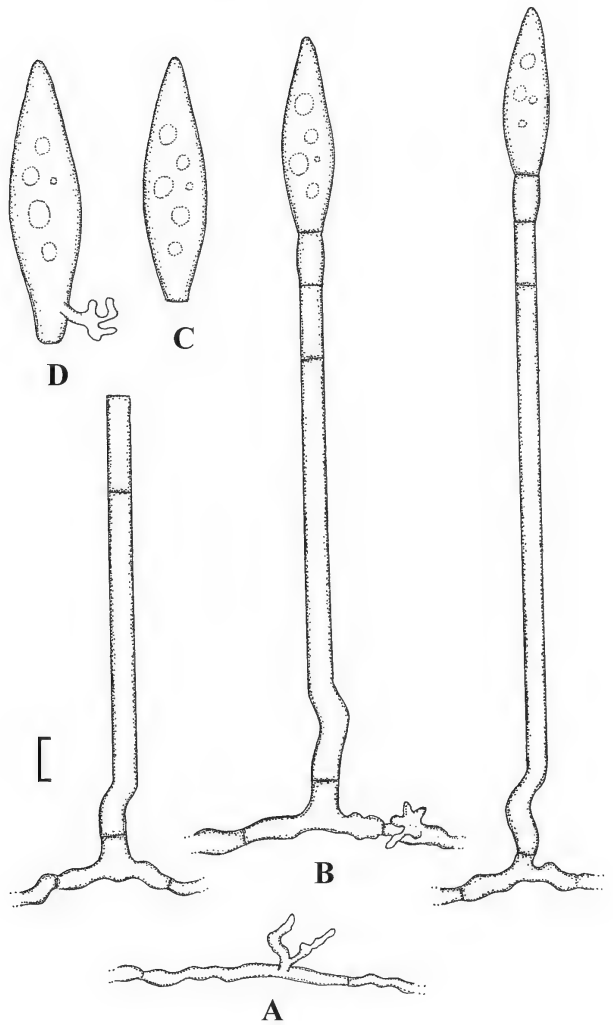

FIGURE 1. *Bougainvillea* powdery mildew.
A: Hypha with appressorium; **B**: Conidiophores; **C**: Conidium; **D**: Conidium with germ tube.
Based on type material [HAL 2588 F]. Bar = 10 µm. U. Braun del.

Taxonomy

***Bougainvillea* powdery mildew** FIG. 1

Mycelium on leaves, hemiendophytic, superficial mycelium mostly hypophyllous, with hyphae emerging through stomata. Hyphae hyaline, septate, thin-walled, 2–6 µm wide, frequently with approximately right angled

branching. Hyphal appressoria numerous, distinct, oblong, hooked, sinuous and/or coral-like. Conidiophores consistently arising from the upper surfaces of superficial hyphae (conidiophores emerging through stomata not observed), and more or less midway between two septa, cylindrical, straight, unbranched, 140–252.5 (average 180) µm long and 7.5–9 µm wide, hyaline, thin-walled, smooth; foot-cells very long, cylindrical, but sinuous to somewhat coiled at the very base just above the basal septum that is slightly elevated above the junction with the mother cell, foot-cells followed by 1–2 much shorter cells. Conidia formed singly, one-celled, dimorphic, primary conidia lanceolate to ovoid-lanceolate, distinctly attenuated towards the apex, 62.5–87.5 (average 77.65) × 12.5–27.5 (average 20.85) µm, secondary conidia ellipsoid-subcylindrical, ends subtruncate, 55–77.5 × 7.5–15 µm, hyaline, with numerous oil-droplets, germ tubes arising near base or apex, single, short, producing hooked and/or coral-like appressoria.

Material examined: **MEXICO, Michoacan, Morelia municipality,** urban garden, on leaves of *Bougainvillea spectabilis* Willd. (*Nyctaginaceae*), September 2009, S. Fernández-Pavía (HAL 2588 F); September 2009, S. Fernández-Pavía (EBUM 23687, GenBank KC556804); **Tarímbaro municipality,** urban garden, on leaves of *B. spectabilis*, July 2009, S. Fernández-Pavía (EBUM 24362).

Discussion

The incidence of powdery mildew on *Bougainvillea* was very low: only four plants, or 1.6%, were infected. This is an atypical powdery mildew because all structures are only sparingly developed and rather inconspicuous, perhaps one reason that this fungus has been overlooked hitherto.

A blast search based on the sequence retrieved from our *Bougainvillea* powdery mildew (KC556804) did not yield any hits and showed only a distant similarity (80–83%) with other sequences representing *Leveillula* G. Arnaud and *Phyllactinia* Lév. These homologies are listed below:

83%: *Leveillula chrozophorae* U. Braun (AB045147, *Chrozophora tinctoria*, Iran), *L. duriaei* (Lév.) U. Braun (AB667859, *Phlomis persica*, Iran), and *L. taurica* s. lat. (AB667864, *Carthamus* sp., Iran).

82%: *L. duriaei* (AB044373, *Salvia nemorosa*, Iran), *L. elaeagni* (Jacz.) Simonyan & Heluta (AB042642, *Elaeagnus orientalis*, Turkmenistan), *L. lactucarum* Durrieu & Rostam (AB044375, *Lactuca serriola*, Iran), *L. lanuginosa* (Fuckel) Golovin (AB044376, AB045153, *Echinophora sibthorpiana* and *Heracleum persicum*, Iran), *L. loranthi* Haajian et al. (AB044377, *Loranthus europaeus*, Iran), *L. thevenotiae* (Jacz.) Golovin (AB044383, *Thevenotia persica*, Iran) as well as several sequences deposited as *L. taurica* s. lat. (AB045108, 667868, 667874, 667882, diverse hosts, Iran; AY912077, *Triglochin maritima*, origin unclear; GQ860947, *Mentzelia laevicaulis*, origin unclear; JQ885445, *Capsicum* sp., South Korea).

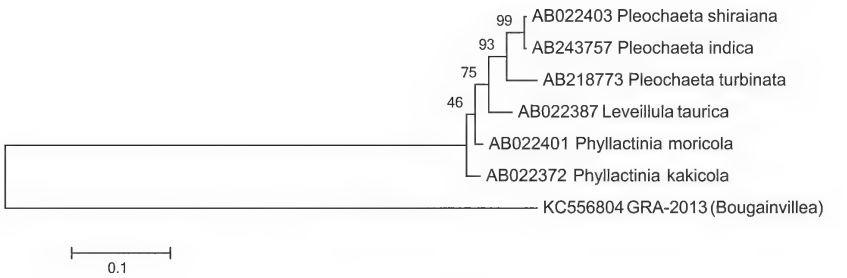

FIGURE 2. *Bougainvillea* powdery mildew. Restricted phylogenetic neighbor-joining tree, prepared by using the program Mega5 with Tajima-Nei distance calculation to demonstrate relationships of this powdery mildew to its closest relatives.

81%: *Phyllactinia fraxini* (DC.) Fuss (AB080513, *Fraxinus longicuspis*, Japan; AB080553, *F. excelsior*, Lithuania; AB080543, *Syringa vulgaris*, Germany).

80%: *P. roboris* (Gachet) S. Blumer (AB080516, *Castanea crenata*, Japan), *Phyllactinia* sp. (AB080546, *Castanopsis* sp., China; AB080564, *Chionanthus virginicus*, Germany).

No sequence agreeing with that of our *Bougainvillea* powdery mildew has yet been deposited in GenBank; our comparisons indicate that within powdery mildews the closest affinity is with species of the tribe *Phyllactinieae* R.T.A. Cook et al. Based on its most significant morphological characters — conidiophores that arise exclusively from superficial hyphae and with sinuous to somewhat coiled foot-cells — this fungus might be assigned to *Ovulariopsis* Pat. & Har., the anamorph of *Phyllactinia* and *Pleochaeta* Sacc. & Speg., as currently circumscribed by Braun & Cook (2012). The formation of dimorphic conidia rather suggest an asexual morph of *Pleochaeta* previously referred to as *Streptopodium* R.Y. Zheng & G.Q Chen, a genus originally introduced for *Ovulariopsis*-like powdery mildew anamorphs characterized by conidiophores with twisted foot-cells and associated with sexual morphs belonging to *Phyllactinia* and *Pleochaeta*.

Braun & Cook (2012) discussed asexual morphs of *Phyllactinia* and *Pleochaeta* in detail. Combinations of conidiophores with coiled foot-cells and formation of dimorphic conidia are not confined to *Pleochaeta* anamorphs, but are also known in various asexual morphs of *Phyllactinia* species, e.g., *P. bauhiniae* Y.S. Paul, *P. cassiae* G.J.M. Gorter & Eicker, and *P. chorisiae* Viégas. There is no real morphological basis for maintaining *Streptopodium* as a separate genus, and Braun & Cook (2012) reduced it to synonymy with *Ovulariopsis,* emending the circumscription of that genus. In addition, various sequences retrieved from

Pleochaeta species were not among those with highest homology (Fig. 2), in that *Pleochaeta* is more distantly related to the *Bougainvillea* powdery mildew than are *Phyllactinia* and *Leveillula*. The formation of dimorphic conidia and the conidial shape agrees well with species of *Oidiopsis* Scalia (anamorphs of *Leveillula*) but in *Leveillula* conidiophores emerge exclusively (or at least partly) through stomata and twisted conidiophore foot-cells are unknown.

In conclusion, we believe that the *Bougainvillea* powdery mildew undoubtedly represents an undescribed species related to genera of tribe *Phyllactinieae*. This powdery mildew is morphologically indistinguishable from the *Ovulariopsis* anamorphs of *Phyllactinia* and *Pleochaeta*, but it cannot be assigned to *Ovulariopsis* due to a conflicting molecular sequence analysis. This analysis confirms a distant relationship to *Leveillula*, *Phyllactinia*, *Pleochaeta*, and *Queirozia* Viégas & Cardoso but excludes it from being congeneric with any of these genera. The introduction of a new anamorph genus, based on molecular data of a single taxon and indistinguishable morphologically from *Ovulariopsis*, is not justified, as it would be of little help in clarifying the taxonomy of this powdery mildew. In the absence of an appropriate genus, it is currently not possible to provide a formal description of the *Bougainvillea* powdery mildew species. The discovery of the teleomorph of this mildew or additional genetically closely allied taxa would be useful, and so further research is necessary.

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Pseudocercospora styracigena sp. nov. on *Styrax* and *Alniphyllum* from China

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Abstract — A new species, *Pseudocercospora styracigena*, is described, illustrated, and discussed. The type specimen is deposited in HMAS.

Key words — cercosporoid fungi, plant pathogen, *Styrax tonkinensis*

Introduction

Pseudocercospora was established in 1910 by Spegazzini. With more than 1500 described species, it is the largest genus of cercosporoid fungi and is distributed predominantly in tropical and subtropical regions. *Pseudocercospora* species are plant pathogens, causing leaf and fruit spot diseases, blights, and fruit rots (Chupp 1954, Deighton 1976, von Arx 1983, Pons & Sutton 1988), and some species are used for biological control purposes against weeds (Den Breeyen et al. 2006). Therefore, *Pseudocercospora* species are an economically significant fungal group. *Pseudocercospora styracigena*, a new species on leaves of *Styrax tonkinensis* collected from Yunnan Province, China, is described, illustrated, and compared with other cercosporoid species on *Styracaceae*.

Taxonomy

Pseudocercospora styracigena Y.L. Guo & B.J. Li, **sp. nov.** Fig. 1

MycoBank MB 808331

Differs from *Pseudocercospora fukuokaensis* by its longer and wider conidiophores, wider conidia, and developed external hyphae.

Type — China, Yunnan Province, Jinghong, on living leaves of *Styrax tonkinensis* (Pierre) Craib ex Hartwich (*Styracaceae*), August 1973, coll. Liu Xi-jin (HMAS 62748, **holotype**).

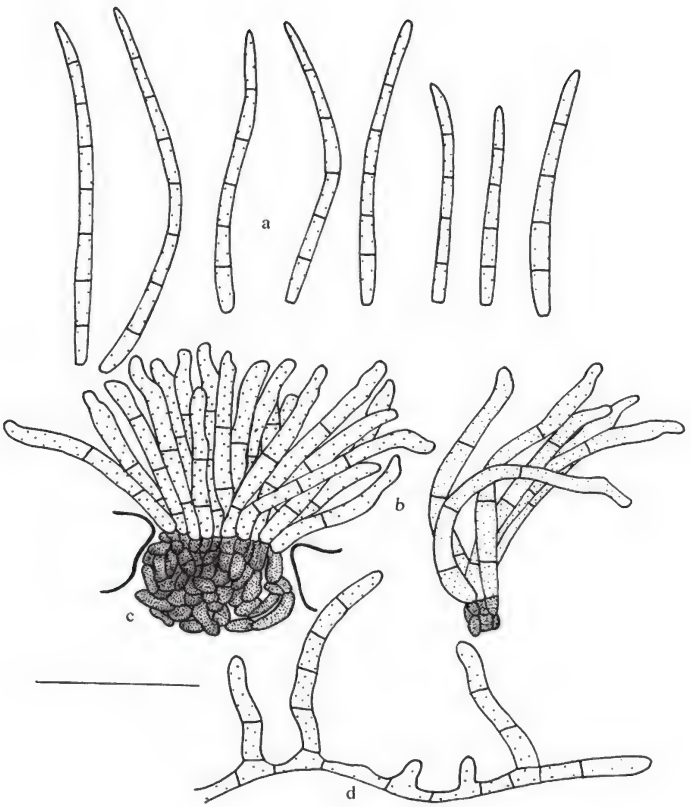

Figure 1. *Pseudocercospora styracigena* (HMAS 62748, holotype).
a. Conidia; b. Conidiophores; c. Stroma; d. External hypha. Bar = 40 μm.

Etymology: Derived from the host genus *Styrax*.

Leaf spots amphigenous, circular to irregular, 0.5–8 mm diam., often confluent, at first gray to grayish brown, later center olivaceous-brown to dark brown, surrounded by a brown to dark brown line on the upper surface, grayish to pale brown on the lower surface. Caespituli amphigenous. Primary mycelium internal; secondary mycelium external, hyphae pale olivaceous to olivaceous, branched, septate, smooth, 2–3.5 μm wide. Stromata substomatal, globose, brown to dark brown, 20–80 μm diam. Conidiophores densely fasciculate or solitary, arising from the external hyphae as lateral branches, olivaceous to moderate olivaceous-brown, uniform in color, irregular in width, straight to curved, branched, geniculate, denticulate, rounded to conical at the

apex, 0–6-septate, sometimes constricted at the septa, 10–110 × 3–5.3 µm. Conidiogenous loci inconspicuous. Conidia cylindrical to cylindrical-obclavate, pale olivaceous to olivaceous, straight to curved, obtuse at the apex, obconically truncate at the base, 2.4–4 µm wide, 3–9-septate, 30–95 × 2.5–5µm.

Additional specimens examined — **China**, **Hunan Province**, Zhangjiajie, on living leaves of *Alniphyllum* sp. (*Styracaceae*), 14 September 1987, coll. Zhao Wen-xia, no. 51 (HMAS 62127, as *Pseudocercospora styracis*); **Yunnan Province**, Jinghong, on living leaves of *Styrax tonkinensis*, 16 July 1976, coll. Han Shu-jin, Zheng Ru-yong, no. 331 (HMAS 62747).

Discussion

Zhao & Guo (1993) previously determined the specimen of *Pseudocercospora styracigena* on *Alniphyllum* sp. from Hunan (HMAS 62127) as *Pseudocercospora styracis* (Chupp) Y.L. Guo & W.X. Zhao [≡*Passalora styracis*], but Crous & Braun (2003: 392) stated that this was a misdetermination of an undescribed species.

Four cercosporoid species have been reported previously on *Styracaceae: Cercospora styracicola* X.J. Liu & Y.L. Guo on *Styrax faberi* Perkins (Liu & Guo 1984); *Passalora halesiicola* U. Braun et al. [≡"*P. halesiae*" nom. inval.] on *Halesia* spp. (Braun et al. 2009, Crous & Braun 2003, Dearness 1941); *Passalora styracis* (Chupp) U. Braun & Crous on *Styrax americana* (Chupp 1954, Crous & Braun 2003); and *Pseudocercospora fukuokaensis* (Chupp) X.J. Liu & Y.L. Guo on *Styrax* spp. (Chupp 1954, Crous & Braun 2003, Guo & Liu 1992, Togashi & Katsuki 1952). *Cercospora styracicola*, *Passalora halesiicola*, and *P. styracis* differ from *Pseudocercospora styracigena* by having different generic characteristics. *Pseudocercospora fukuokaensis* differs from *P. styracigena* by its shorter and narrower conidiophores (5–30 × 2.5–4 µm) and narrower conidia (30–70 × 2–3.5µm) as well as in lacking external hyphae (Togashi & Katsuki 1952, Chupp 1954).

Acknowledgments

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Neozygites species associated with aphids in Chile: current status and new reports

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Abstract —Three species of *Neozygites* were recorded during a 2007–13 survey of the occurrence of the genus on aphids in Chile. *Neozygites osornensis* is known from recent studies, and *N. fresenii* and *N. cinarae* are reported as new records for Chile. *Neozygites lageniformis*, which was not found during the survey, had been recorded previously in Chile. Morphological descriptions, symptoms on infected insects, host spectrum, and geographical distribution of all four species are presented, and a key to *Neozygites* species associated with aphids in Chile is included. These fungi, which are important natural enemies of aphids, may be considered for future aphid pest control.

Key words — entomopathogenic fungi, aphid pathogens, *Entomophthoromycota*

Introduction

Insect-pathogenic fungi represent a phylogenetically heterogeneous group of microorganisms. However, a majority of the economically important species belongs to the phyla *Ascomycota* (order *Hypocreales*) and *Entomophthoromycota*. In general, these fungi are considered excellent candidates for the biological control of various insect pests (Latgé & Papierok 1988, Charnley & Collins 2007, Roy et al. 2010). The hypocrealean species are well studied and some of them are

commercially used with success in various strategies of biological control (Shah & Pell 2003, Sandhu et al. 2012). While species in the *Entomophthoromycota* have been used successfully in the laboratory, they have not yet been commercially formulated into an applicable mycoinsecticide (Barta & Cagáň 2006a), although they have been introduced and spread successfully as an inoculum (Milner et al. 1982, Elkinton et al. 1991). Conservation biological control has been used for other fungi in *Entomophthoromycota,* and there have been attempts in the United States of America to suppress mite populations in soybean crops with fungal pathogens rather than insecticide treatments (Klubertanz et al. 1991).

Neozygites (*Neozygitales*, *Neozygitaceae*) (Humber 2012) comprises a relatively homogenous group of 18 species exclusively pathogenic to arthropods (*Acari*, *Hemiptera*, and *Thysanoptera*) (Delalibera et al. 1992, Montserrat et al. 1998, Shah & Pell 2003, Keller & Petrini 2005, Keller 2007, Wekesa et al. 2010). *Neozygites* species are important natural enemies that may control arthropod populations under microclimatic conditions favorable for the fungus (Latgé & Papierok 1988; Barta & Cagáň 2003, 2006a; Klingen & Westrum 2007; Castro et al. 2013). Generally, most *Neozygites* species can be divided into two subgroups (*Neozygites fresenii*-type or *Neozygites floridana*-type) based on their morphologies and life cycles (Keller 1997, 2006; Delalibera et al. 2004). Their narrow host specificity makes them valuable control agents that present a negligible threat to non-target organisms (Barta 2004). Although the genus has a worldwide distribution, some *Neozygites* species are known only from their type localities. As there is limited literature about the genus in Chile (Aruta et al. 1984; Aruta & Carrillo 1989; Montalva et al. 2010, 2013), our goal was to review and update knowledge on *Neozygites* species associated with aphids in Chile.

Materials & methods

The literature was searched for publications on *Neozygites* associated with aphids in South America with an emphasis on Chile. During 2007–13, aphid populations were observed for *Neozygites* in 25 localities of southern Chile (36°49′41.5″–53°10′00″S 70°56′00″–73°03′04.93″W).

Dead aphids were collected in the field. Black cadavers filled with internally produced resting spores were either examined immediately after being taken to the laboratory or stored at 4 °C until examination. Brown cadavers filled with vegetative cells and hyphal bodies were incubated at 25 °C in moist chambers (plastic containers and moistened filter paper) to induce external conidiogenesis. *Neozygites* species were identified based on morphology according to Keller (1991, 1997).

Mounts were prepared in lactophenol-aceto-orcein (LPAO) as described by Keller (1987). Fungal structures were examined with a Nikon Eclipse

E600 microscope, photographed with a Nikon DS-Fi1 digital camera at a magnification of 1500×, and measured with Motic Images Plus 2.0 software. At least four series of measurements were taken for taxonomically important microstructures. Each series represented 50 measurements, and for each series a mean value was calculated. A final value of each dimension consisted of two ranges: the range of mean values of all series and the range of extreme values of all measurements (in parentheses).

Vouchers of the newly recorded species were conserved in Laboratorio Regional, Servicio Agrícola y Ganadero, Osorno, Chile (LA-N).

Results

Current status of aphid-pathogenic *Neozygites* species in Chile

Neozygites lageniformis was not found during this survey but has been recorded previously in Chile. Two of the three aphid-pathogenic *Neozygites* species observed during the survey are presented as first records from this country. Table 1 provides morphometric characteristics of all *Neozygites* species recorded in Chile. Detailed data on each species are presented below.

Taxonomic descriptions

Neozygites cinarae S. Keller, Sydowia 49: 137. 1997. Plate 1

Hyphal bodies not observed. Primary conidia ovoid to pyriform, formed apically on unbranched conidiophores, with papilla distinct, truncate or slightly rounded, 25.7–26.8 × 18.4–19.5 µm. Forcibly discharged secondary conidia were only rarely observed; capilliconidia are short almond-shaped with a terminal mucoid droplet, brownish, produced apically on slender capillary, 26.0–30.3 × 11.5–12.8 µm. Zygospores ellipsoidal, black, with smooth surface, 33.8–35.3 × 24.3–24.7 µm.

Specimen examined: on *Cinara cedri* Mimeur: **CHILE**, Valdivia, 39°48′18″S 73°15′10″W, March 2013, by C. Montalva (LA-N15032013).

Ecology & occurrence: Infected aphids were found fixed by their probosces on the underside of branches of *Cedrus atlantica* (Endl.) Manetti ex Carrière. Almost all aphids (> 90%) sampled from March to May 2013 were infected and brown or black when filled with resting spores. This is the first record for Chile.

Neozygites fresenii (Nowak.) Remaud. & S. Keller, Mycotaxon 11: 332. 1980. Plate 2

Hyphal bodies spherical to subspherical, 13.0–13.9 µm in diameter. Conidiophores unbranched. Primary conidia spherical, 21.0–21.7 × 15.9–16.3 µm; papilla distinct, cylindrical, blunt to slightly rounded. Secondary conidia (if discharged) like primary conidia on short unbranched conidiophores; capilliconidia passively dispersed, almond-shaped, apical on slender

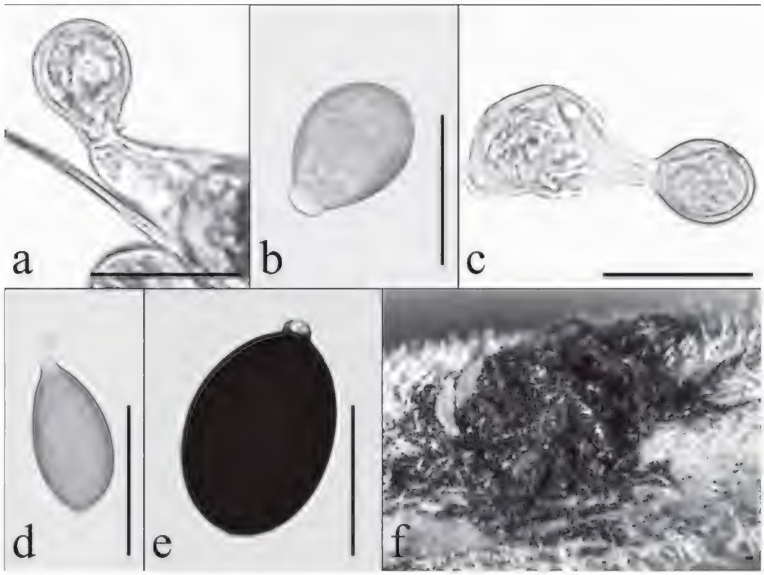

Plate 1. *Neozygites cinarae* on *Cinara cedri*. a. Conidiophore forming primary conidium. b. Primary conidium. c. Formation of secondary conidium. d. Capilliconidium. e. Resting spore. f. *Cinara cedri* infected with *Neozygites cinarae*. Bars = 25 µm.

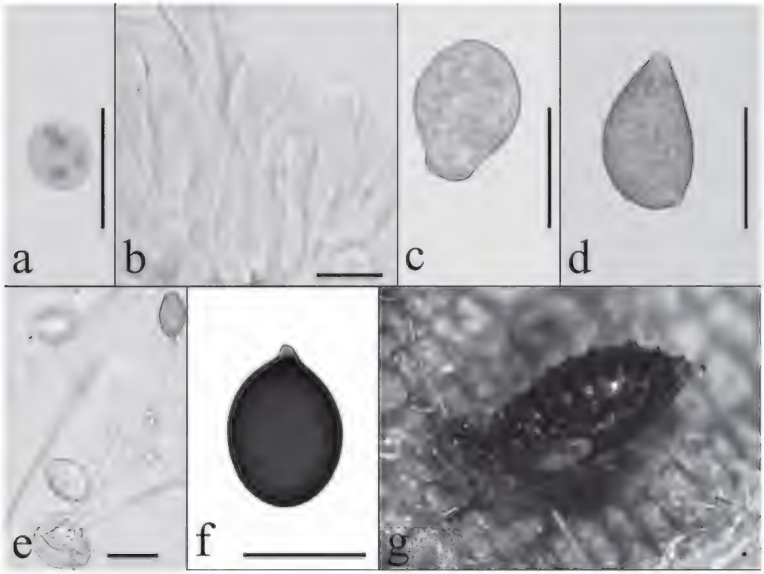

Plate 2. *Neozygites fresenii* on *Myzocallis coryli*. a. Hyphal body with visible nuclei. b. Unbranched conidiophores. c. Primary conidium. d. Capilliconidium. e. Formation of capilliconidia. f. Resting spore. g. *Myzocallis coryli* infected with *Neozygites fresenii*. Bars = 20 µm.

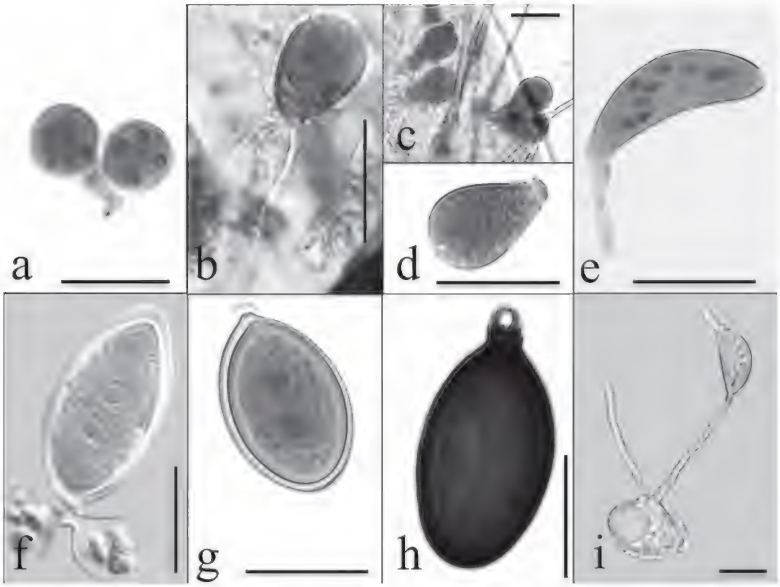

PLATE 3. *Neozygites osornensis* on *Cinara cupressi*. a. Subspherical hyphal bodies with visible nuclei. b. Conidiophore forming primary conidium. c. Primary conidia attached to aphid leg. d. Primary conidium. e. Crescent-shaped capilliconidium with visible nuclei. f. Zygospore with visible nuclei developing by conjugation of two hyphal bodies containing degenerating nuclei. g. Immature zygospore. h. Fully developed zygospore. i. Young germ capilliconidium produced by zygospore on long capillary germ conidiophore. Bars = 20 µm.

capilliconidiophores, 22.8–24.3 × 12.8–13.2 µm. Zygospores broadly ellipsoidal, thick-walled, episporium black, smooth, 26.1–28.4 × 18.7–20.4 µm.

SPECIMENS EXAMINED: on *Myzocallis coryli* (Goetze): **CHILE**, Puyehue, 40°38′19″S 72°40′55″W, 2012, by C. Montalva (LA-N25032012).

ECOLOGY & OCCURRENCE: Infected aphids fixed by their probosces and/or legs were found on the underside of branches of the common hazel (*Corylus avellana* L.); grayish brown to brick or dark brown when conidia were formed, and black when resting spores were present. About 30% of the aphids sampled from February to April 2012 were infected. This is the first record for Chile.

Neozygites osornensis Montalva & Barta, Mycologia 105: 663. 2013. PLATE 3

Hyphal bodies spherical or subspherical, 16.4–16.9 µm in diameter. Primary conidia form apically on unbranched conidiophores, ovoid to pyriform, with papilla truncate or slightly rounded, 20.8–23.8 × 13.6–15.9 µm. capilliconidia apical on long slender capillary conidiophores, elongate, crescent-shaped, 28.0–28.5 × 8.5–8.8 µm. Zygospores ellipsoidal, with episporium smooth, dark-brown to black, 31.8–40.8 × 21.5–22.7 µm; germinating by forming capillary

Table 1. Morphometric analysis of taxonomically important structures of four different *Neozygites* species on aphids in Chile.

Fungal structures	*N. cinarae*	*N. fresenii*	*N. lageniformis*	*N. osornensis*
Hyphal bodies (diam.; µm)	No data	13.0–13.9 (4 series)	No data	16.4–16.9 (4 series)
Nuclei (diam.; µm)	No data	2.60–2.84 (4 series)	No data	3.16–3.36 (4 series)
Conidiophores	Unbranched	Unbranched	No data	Unbranched
Primary conidia (µm)				
Length (L)	25.7–26.8 (4 series*)	21.0–21.7 (4 series)	28.4–32.2	20.8–23.8 (4 series)
Diameter (D)	18.4–19.5 (4 series)	15.9–16.3 (4 series)	17.3–21.5	13.6–15.9 (4 series)
L/D	1.4–1.4 (4 series)	1.33–1.36 (4 series)	No data	1.48–1.52 (4 series)
Capilliconidia (µm)				
Length (L)	26.0–30.3 (4 series)	22.8–24.3 (4 series)	30.0–33.6	28.0–28.5 (4 series)
Diameter (D)	11.5–12.8 (4 series)	12.8–13.2 (4 series)	17.7	8.5–8.8 (4 series)
L/D	2.23–2.37 (4 series)	1.79–1.85 (4 series)	No data	3.23–3.36 (4 series)
Number of nuclei	4 (3–6) (n= 7**)	No data	No data	7 (5–10) (n= 20)
Capillary tube length (µm)	No data	No data	No data	30.5–31.8 (2= series)
Resting spores (µm)				
Length (L)	33.8–35.3 (4 series)	26.1–28.4 (4 series)	No data	31.8–40.8 (4 series)
Diameter (D)	24.3–24.7 (4 series)	18.7–20.4 (4 series)	No data	21.5–22.7 (4 series)
L/D	1.37–1.43 (4 series)	1.39 (4 series)	No data	1.47–1.85 (4 series)
Rhizoids	Absent	Absent	Absent	Absent
Reference	This paper	This paper	Aruta & Carrillo 1989	Montalva et al. 2013

* Series = one series represents 50 measurements; the range consists of mean extreme values of series.

**n = number of measurements.

conidiophores, averaging 72.4 µm long (n: 43), with apical germ capilliconidia, 28.0 × 12.4 µm (n: 45) (Montalva et al. 2013).

Specimens examined: on *Cinara cupressi*: **CHILE**, Osorno, 40°35′14″S 73°05′38″W, 4 April 2007, by C. Montalva (SGO 161 401, holotype).

Ecology & occurrence: Infected aphids can be easily recognized hanging on twigs and by an obvious change of their color during sporulation (turning from yellowish-brown to reddish-brown). During the infection progress, aphids gradually became swollen and turgid. Cadavers that were reddish-brown during the formation of external conidia became black when internal zygospores developed. Cadavers usually hung and remained with their probosces inserted in the branches. After certain time the cadavers either dried up and fell down to the soil or liquefied and disintegrated during zygosporogenesis, thus dispersing the resting spores on the bark of twigs. More than 80% of aphids sampled from November to December 2013 in Osorno were infected (Montalva et al. 2013).

Key to aphid-pathogenic *Neozygites* species from Chile

1. Observed on *Myzocallidinae* or *Aphidinae* 2
1. Observed on *Lachninae* or *Pterocommatinae* 3
2. Primary conidia average length ≤22 µm; capilliconidia averaging < 25 µm, with L/D 1.8 ..... *Neozygites fresenii*
2. Primary conidia average length ≥29 µm; capilliconidia averaging ≥ 30 µm, with L/D 2.0 ..... *Neozygites lageniformis*
3. Primary conidia average length 26 µm; capilliconidia almond-shaped, average diameter >11 µm ..... *Neozygites cinarae*
3. Primary conidia average length 23 µm; capilliconidia crescent-shaped, average diameter <9 µm ..... *Neozygites osornensis*

Discussion

Dead insects and mites killed by different *Neozygites* species have been reported from several South American countries (Humber et al. 1981, Agudelo-Silva 1986, Löhr et al. 1990, Gómez de Picho 1991, Delalibera et al. 1997, 2000, Van der Geest et al. 2002, Scorsetti & López-Lastra 2007, Roggia et al. 2009). Previously, however, *Neozygites* species pathogenic to aphids have been recorded only in Argentina and Chile (Scorsetti et al. 2007, Sosa-Gómez et al. 2010, Montalva et al. 2013). The first reports from Chile of any *Neozygites* species were made in the 1980s, when *N. parvispora* (D.M. MacLeod & K.P. Carl) Remaud. & S. Keller (on thrips) and *N. lageniformis* (on aphids) were observed infecting natural arthropod populations (Aruta et al. 1984, Aruta & Carrillo 1989). *Neozygites parvispora* is a widespread fungal parasite of various thrips species with a primarily European distribution (Bałazy 1993, Keller 1997). *Neozygites lageniformis* is rare and incompletely described species from the

New World (Thaxter 1888, Aruta & Carrillo 1989), previously observed only twice (in aphids in United States of America and Chile). During the survey we did not find this fungus in *Myzocallis coryli* colonies. Although there are no complete data available on its morphology, *N. lageniformis* is still regarded as a separate species (Bałazy 1993). However, until additional records and a more accurate description become available, its taxonomic status is uncertain.

Neozygites cinarae is a fungal pathogen of aphids in the genus *Cinara*. Previously reported from Europe on *C. pilicornis* (Hartig) and *C. piceae* (Panzer) (Keller 1997, Barta et al. 2005, Barta & Cagáň 2006b, Montalva et al. 2013), ours is first report for South America. In Chile, *N. cinarae* was found only in *C. cedri* colonies on the host tree, *Cedrus atlantica*. No data are available about its use as a biological control in laboratory or field conditions. Its high incidence during our survey suggests that during autumn *N. cinarae* could be a good natural regulator of *C. cedri* populations along with other natural enemies such as coccinellid beetles and syrphid flies.

Neozygites fresenii is a widely occurring fungal pathogen of aphids. It has been reported from Europe (Keller 1991, Bałazy 1993, Barta & Cagáň 2002), North and South America (Steinkraus et al. 1995, Scorsetti et al. 2007), South Africa (Hatting et al. 1999), the South Pacific (Keller 1997), and Australia (Milner & Holdom 1986). Although the fungus is reported to have a broader host spectrum, in Chile *N. fresenii* was found only in *Myzocallis coryli* colonies. In Argentina, *N. fresenii* has also been identified from *Brevicoryne brassicae* L., *Myzus* sp., and *Aphis fabae* Scop. in Buenos Aires province (Scorsetti et al. 2007). *Neozygites fresenii* often causes widespread epizootics in aphid populations (Steinkraus et al. 1995, Keller 1997, Barta 2004). While this fungus is considered to be best adapted to hot, humid, and even tropical conditions (Remaudière 1977, Keller 1997), epizootics have also been reported in Iceland (Austarå et al. 1997, Nielsen et al. 2001). The epizootiology of *N. fresenii* in annual crop ecosystems has been well studied (Steinkraus et al. 1995, 1996, Barta & Cagáň 2002), but its commercial exploitation is limited due to absence of in vitro isolates. In Arkansas (USA), a method for mass-harvesting *N. fresenii* from natural epizootics in *Aphis gossypii* populations in a commercial cotton field was developed with the intention of using the collected inoculum for targeted aphid control (Steinkraus & Boys 2005).

Neozygites osornensis, first described during a survey of natural enemies of invasive *Cinara cupressi* colonies in Chile (Montalva et al. 2013), was also found in five localities in southern Chile on *C. cupressi* and *C. tujafilina*, which usually co-occurs with *C. cupressi*. The infection usually occurred between November (end of spring) and March (end of summer). We suggest that *N. osornensis* is a natural enemy that could serve as a biological control of *C. cupressi* in Chile. *Neozygites osornensis* might also be a candidate for conservation biological

control of *C. tujafilina,* an aphid attacking a native host tree, *Austrocedrus chilensis* (D. Don) Pic. Serm. & Bizzarri, whose populations are now defined as vulnerable (Hechenleitner et al. 2005). The application of chemical insecticides to control *C. tujafilina* is forbidden in national parks (Corporación Nacional Forestal, CONAF) without permission (CONAF 2006).

All *Neozygites* species that we found in Chile produce resting spores that allow the fungi to persist during unfavorable conditions for several years (Hajek 1997) and, thereby, to serve as a reservoir of infection in the ecosystem. For *Neozygites* collected during the surveys, we usually found capilliconidia on aphid cadavers diseased by *N. cinarae* and *N. fresenii* during March (end of summer). On the other hand, *N. osornensis* capilliconidia were less frequently observed and were obtained only in the laboratory in a humid chamber on infected *Cinara cupressi* collected in Osorno during November (end of spring). Capilliconidia of *Neozygites* spp., which are usually produced during the vegetation period, are responsible for disseminating infection among host populations (Keller 1997). Contrary to what Keller reported (1997), we observed forcibly discharged secondary conidia of *N. cinarae* in Valdivia, although this conidial type was found only rarely. The absence of rhizoids and pseudocystidia for all species discussed here agrees with Keller (1991, 1997) and Bałazy (1993). Very few studies have been published for any location treating natural occurrences of entomophthoroid fungi infecting aphids feeding on woody plants. However, it appears that *N. fresenii, Entomophthora planchoniana* Cornu, and *Zoophthora aphidis* (Hoffm.) A. Batko are the most frequent fungal species associated with tree-dwelling aphids (Nielsen et al. 2007, Barta 2009); these findings are reinforced by our findings about *N. fresenii.*

The identification of these fungi is an important step for a better knowledge of natural fungal enemies of aphids in Chile and throughout the world. However, more studies are on virulence, culturing, strain characterization, and molecular identification are needed to bring greater and much needed clarity to our understanding of these relatively little known fungi.

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First report of *Pseudoveronaea ellipsoidea* causing sooty blotch and flyspeck in China

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Abstract — *Pseudoveronaea ellipsoidea* is reported and described as a newly recorded species from China. This fungus was isolated from peels of hawthorn and apple fruit collected from Shaanxi and Shanxi Provinces, and caused sooty blotch and flyspeck signs on these hosts. Based on morphological characteristics and phylogenetic analysis, we identified our isolates as *P. ellipsoidea*. This is also the first report of the genus *Pseudoveronaea* in China.

Key words — *Dissoconiaceae,* taxonomy, morphology, phylogeny

Introduction

Sooty blotch and flyspeck (SBFS) fungi colonize the waxy cuticle of a wide range of plant hosts in humid regions worldwide. They cause cosmetic damage to cultivated fruits and ornamental plants due to the smudges formed on the surface, resulting in economic losses to growers (Williamson & Sutton 2000, Batzer et al. 2005, Gleason et al. 2011). For nearly 75 years this disease was considered to be two different diseases, each caused by a single species (Colby 1920, Williamson & Sutton 2000). Based on analysis of rDNA sequences combined with traditional morphology, researchers obtained a better understanding of SBFS, the number of species in the complex increased to more than 80 (Díaz Arias et al. 2010; Frank et al. 2010; Gleason et al. 2011; Li et al. 2011, 2012), and many new genera and species were described in the discovery process.

Among these fungi, *Pseudoveronaea* was recently established as a new genus and named after its morphological similarity to *Veronaea* (Li et al.

2012). A member of the family *Dissoconiaceae*, *Pseudoveronaea* is nevertheless phylogenetically and morphologically distinct from other genera in this family, in that it produces only one type of conidia and these are septate. Currently there are only two species in this genus, *P. ellipsoidea* and *P. obclavata*, both associated with SBFS on apple (Gleason et al. 2011, Li et al. 2012).

In our recent study on SBFS, four isolates obtained from hawthorn and apple were identified as *P. ellipsoidea,* based on morphological comparison and phylogenetic analysis of the ITS region.

Materials & methods

Isolates and morphology

Hawthorn (*Crataegus pinnatifida* Bunge) and apple (*Malus domestica* Baumg.) fruit with SBFS signs were collected from Shaanxi and Shanxi Provinces in 2011. Peels with SBFS were cut off, examined and photographed under the dissecting microscope, then pressed between paper towels until dry and preserved. Pieces of SBFS colonies on the peels were transferred to potato-dextrose agar (PDA) and cultured at 25°C in the dark (Sun et al. 2003). After one month, pure isolates were obtained and transferred to synthetic nutrient-poor agar (SNA) for morphological examination and description using the method of Zhang et al. (2009). Colony descriptions were based on cultures on PDA and oatmeal agar (OA) after one month in the dark at 25°C. Specimens of representative dried cultures were deposited in the Fungal Herbarium of Northwest A&F University, Yangling, Shaanxi Province, China (HMUABO).

DNA extraction, PCR and sequencing

Genomic DNA was extracted from fungal mycelium according to the protocol of Barnes et al. (2001). The primers ITS1-F (Gardes & Bruns 1993) and ITS4 (White et al. 1990) were used to amplify the internal transcribed spacer (ITS) region of nuclear ribosomal DNA. The PCR conditions followed the methods of Zhuang et al. (2010). PCR products were sequenced by Sangon Biotech (Shanghai), China.

Sequence alignment and phylogenetic analysis

Sequences in this study and other sequences downloaded from GenBank were put into CLUSTAL X to perform preliminary alignment (Thompson et al. 1997), and then manually adjusted in BioEdit v. 5.0.9.1 (Hall 1999). Maximum parsimony (MP) analysis of aligned sequences was conducted using PAUP v. 4.0b10 (Swofford 2003). Heuristic searches were performed with a 1000 random taxa addition and tree bisection-reconnection (TBR) as the branch-swapping algorithm. All characters were unordered and given equal weight and gaps were treated as missing data. The robustness of clades and internal branches was evaluated by 1000 bootstrap replications. Tree length (TL), consistency index (CI), retention index (RI) and rescaled consistency index (RC) were also calculated. Sequences generated in this study were deposited in GenBank. Alignment and the representative tree were deposited in TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:S13736).

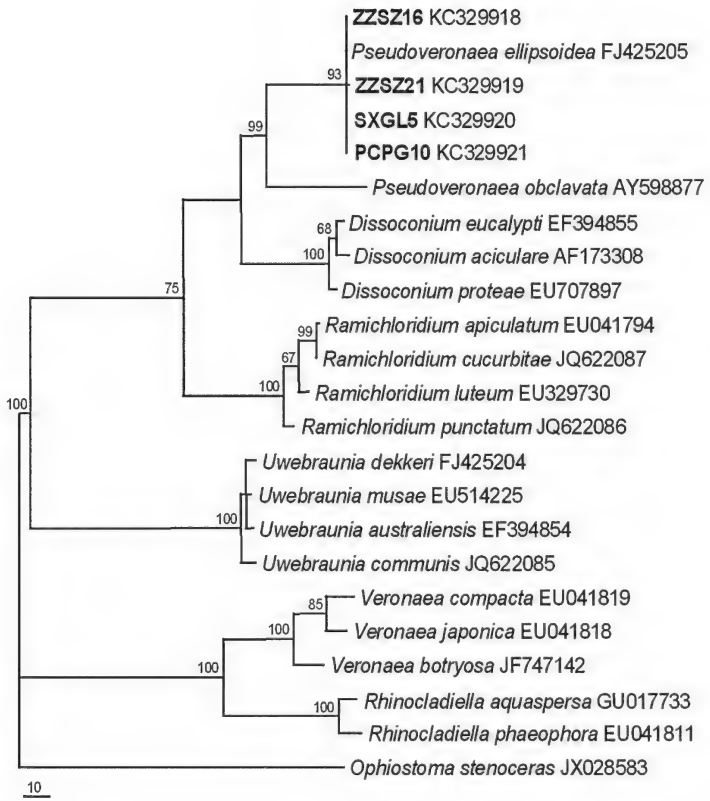

FIG. 1. One of four equally most parsimonious trees obtained from a heuristic search with 1000 random taxon additions of the ITS sequence alignment. The bootstrap support values (>50%) based on 1000 replicates are shown at the nodes. The tree was rooted to *Ophiostoma stenoceras*. The scale bar shows 10 changes. Strains investigated in this paper are presented in bold.

Results

Phylogenetic analysis

The manually adjusted ITS alignment contained 23 sequences (including the outgroup sequence) and 604 characters including alignment gaps. Of these characters, 223 were parsimony-informative, 102 were variable and parsimony-uninformative, and 279 were constant. Four equally most parsimonious trees were obtained from the analysis, the first of which is shown in FIG. 1 (TL = 601 steps, CI = 0.7920, RI = 0.8808, RC = 0.6976). Two major clades were resolved from the analysis. One clade included five species in *Veronaea* and

Rhinocladiella. The other clade, with 100% bootstrap support, contained 17 strains in *Pseudoveronaea, Dissoconium, Ramichloridium,* and *Uwebraunia.* Our strains clustered together with *P. ellipsoidea* in the first subclade with a bootstrap value of 93%, indicating that they might represent the same species.

Taxonomy

Pseudoveronaea ellipsoidea Batzer & Crous, Persoonia 28: 119, 2012 FIG. 2

HYPHAE septate, branched, smooth, hyaline and turning to brown as culture aged, 2–3.5 µm diam. CONIDIOPHORES erect, solitary, arising from aerial hyphae, straight to flexuous, unbranched, 3–11-septate, subcylindrical, smooth, thick-walled, brown, 35–115 × 3–4.5 µm. CONIDIOGENOUS CELLS terminal, integrated, subcylindrical, with apical taper to acutely rounded apex, pale brown to brown, 6–21 × 3–5 µm, sympodially proliferating, rachis straight or flexuous, scars slightly darkened and unthickened, 0.5–1 µm diam. CONIDIA solitary, 0–1-septate, ellipsoid to obclavate, hyaline to pale brown, (6–)8–11 (–16) × 3–4 µm, base truncate, slightly darkened and thickened, 1–1.5 µm diam.

CULTURAL CHARACTERISTICS — Colonies after 1 month on PDA at 25°C flat, raised at the middle, slightly folded, with lobate, smooth margin and sparse aerial mycelium, surface iron-gray, reverse black, reaching (18–)20(–23) mm diam. On OA faster growing, flat, spreading, with lobate, feathery margin and moderate aerial mycelium, surface olivaceous gray, reverse black, reaching 32 mm diam.

On peels of hawthorn fruit, showing the ridged honeycomb mycelial type characterized by clumps and ridges of mycelia, olivaceous gray, without distinct edges. On peels of apple fruit, forming the fuliginous mycelial type characterized by uniform mats of mycelia with distinct, feathered edges.

SPECIMENS EXAMINED: **CHINA, SHAANXI PROVINCE**: Xi'an City, Zhouzhi County, on fruit surface of hawthorn (*Crataegus pinnatifida*), 15 Oct. 2011, Chen C & Dang JL ZZSZ16 (HMUABO; GenBank KC329918), ZZSZ21 (HMUABO; GenBank KC329919); Weinan City, Pucheng County, on fruit surface of apple (*Malus domestica*), 25 Sept. 2011, Li WH & Gao L PCPG10 (HMUABO; GenBank KC329921); **SHANXI PROVINCE**: on fruit surface of apple (*Malus domestica*), 16 Oct. 2011, Li WH & Gao L SXGL5 (HMUABO; GenBank KC329920).

Discussion

Based on phylogenetic analysis and morphological characteristics, we identified our isolates as *P. ellipsoidea*, a newly recorded species for China. This is also the first time that the genus *Pseudoveronaea* has been reported from China.

Previous study showed *P. ellipsoidea* causing SBFS on apple as displaying the fuliginous mycelial type (Li et al. 2012). In our study, *P. ellipsoidea* formed the

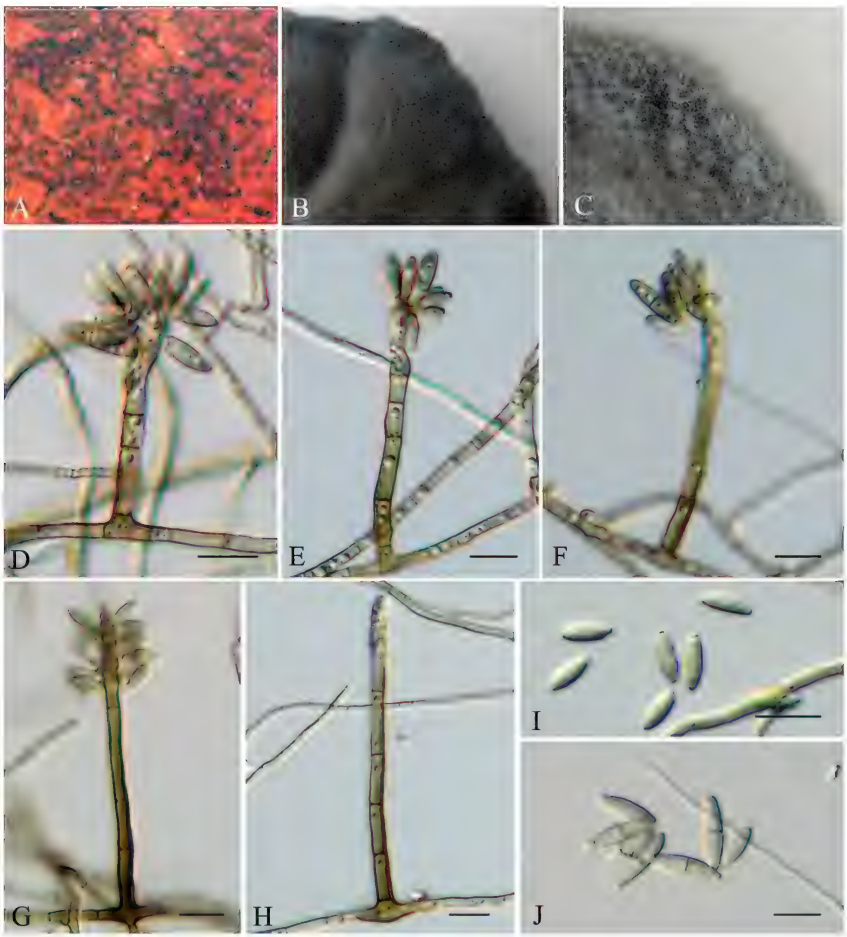

Fig. 2. *Pseudoveronaea ellipsoidea* (ZZSZ16). A. Signs on hawthorn. B. Colony on PDA. C. Colony on OA. D–H. Conidiophores showing rachis, scars and conidia. I–J. Conidia. Bars: D–J = 10 μm.

same mycelial type on apple, but caused a different type, ridged honeycomb, on hawthorn. Hemnani et al. (2008) also found a similar phenomenon in that the same SBFS species produced different mycelial types on different hosts. More investigations are needed to clarify the relationships between hosts and SBFS mycelial types.

So far, only two *Pseudoveronaea* species have been reported in USA (Li et al. 2012) and China. More collections and reports on the genus are required to help us understand the taxonomy, biogeography, and ecology of this genus.

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Molecular annotation of type specimens of *Russula* species described by W.A. Murrill from the southeast United States

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Abstract — Twenty-five historical type collections of North American *Russula* species described by W.A. Murrill were sequenced and analyzed for placement in a phylogenetic and taxonomic framework. Molecular data was successfully obtained from sixteen type specimens (66%), of which eight species have never before been placed in an infrageneric classification system. Classifications and synonymization of taxa proposed by Rolf Singer and others are evaluated. *Russula albimarginata* and *R. emeticiformis* are suggested as synonyms of *R. vinacea,* a widespread and common species in the eastern United States. Four taxa of unknown classification (*R. albimarginata, R. pinophila, R. subcremeiceps, R. subrubescens*) have been classified to subsection level, and an alternative classification is proposed for *R. westii*. A morphological comparison of a collection from British Columbia (Canada) with 99% sequence similarity to *R. levyana* in the Internal Transcribed Spacer 1 (ITS1) region indicates that this region alone may be inadequate for species barcoding in *Russula*.

Key words —phylogenetics, TENN herbarium, high performance DNA extraction, Florida

Introduction

Between 1938 and 1948, W.A. Murrill described 110 species in the genus *Russula* Pers. almost exclusively from the area surrounding Gainesville, Florida (Mycobank 2014). Of the 334 *Russula* species described from the United States, Murrill's work comprises the largest number described by a single mycologist (Buyck 2007). Unfortunately, most of the taxa are poorly known, due partly to Murrill's short diagnoses that focused on macromorphological characters and partly to the lack of extensive studies of *Russula* in the southeast U.S. One notable exception to the lack of attention given to *Russula* in the region is a series of type studies by Hesler (1960, 1961). These studies treat 191 *Russula* species, including 87 species described by Murrill, and they contribute data from microscopic descriptions of the pileus cuticle, lamellar cystidia, and

spore morphology with some discussion of taxonomic affinities proposed by Rolf Singer. Hesler received authentic and type material from major herbaria (FH, MICH, NY, NYS, and FLAS) for these studies, and in the case of Murrill's collections, many were sent as isotypes to be accessioned at TENN (herbarium abbreviations per Thiers 2013).

Central to our current understanding of *Russula* in North America are the studies of Rolf Singer (Singer 1986), which include the most comprehensive infrageneric classification system to include North American taxa. Singer treated a number of Murrill's taxa in various editions of "The *Agaricales* in Modern Taxonomy" (Singer 1951, 1975, 1986), and it is my objective to test these proposed relationships in a phylogenetic context. A recent initiative to revive the taxonomy of North American *Russula* has led to a number of published type studies using advanced modern microscopy and morphometrics and unpublished phylogenies upon which a number of taxonomic affinities have been proposed (Adamčík & Buyck 2010, Adamčík & Buyck 2011a, Adamčík & Buyck 2011b, Adamčík et al. 2013, Buyck & Adamčík 2011a, Buyck & Adamčík 2011b, Buyck & Adamčík 2013). With these renewed concepts, new reports of historical *Russula* taxa are emerging (Adamčík et al. 2010, Buyck et al. 2008, Buyck et al. 2011). The only other comprehensive treatment of a majority of Murrill's taxa can be found in the appendix of Kibby & Fatto (1990), who coded features of southern species for use in their synoptic key system, which also included abbreviated species descriptions. The objective of this study is to contribute to the revival of *Russula* taxonomy in North America by determining whether Murrill's type material at TENN can be molecularly annotated and by exploring what sort of taxonomic inferences can be drawn from these data.

Molecular methods

Following an inventory of *Russula* type material deposited at TENN, 25 type collections of species designated by W.A. Murrill were selected for DNA extraction and molecular annotation (Table 1). In most cases, it was determined that ample material was available to allow using 50 mg of dried material for DNA extraction. The sporocarp material and a pinch of sterile sand were placed in a mortar to which liquid nitrogen was added. The frozen material was ground to a fine powder with a pestle and scraped into a 1.5 mL microtube with a metal spatula. DNA extraction protocols followed the E.Z.N.A. HP Fungal DNA Kit (Omega Bio-Tek, Norcross, Georgia) with the following notable exceptions to improve end-product DNA concentration: 1) 10 µL 2-mercaptoethanol was added to the samples in buffer, and they were allowed to incubate at 65°C for 24 hours (vortexing at the beginning of incubation and intermittently during the final 30 minutes). 2) After the prescribed 300 µl of supernatant and associated reagents were centrifuged through the HiBind DNA column, the remaining supernatant was run through a second round of centrifugation. 3) Two rounds of DNA elution using 50 µL of Elution buffer were performed after a 5 minute incubation at 65°C with the buffer added. Dilutions of 1:10 were made from the genomic DNA product.

TABLE 1. Sequence data from *Russula* taxa selected for phylogenetic comparison with Singer's infrageneric classification

COLLECTIONS[1]	TYPE DESIG.	COLL. #	TENN #	ITS1	ITS2	GENBANK MATCH	INFRA. CLASS.	PHYLO. CLASS.	GENBANK #
R. westii	Isotype	F16404	21262	✓	✓	93% *R. aeruginea* CAN	*Lilaceinae*	*Subcompactinae*	KF810121
R. brunneipes	Isotype	F19537	21229	✓		98% *R.* sp. MA	unknown	uncertain	KF810122
R. subrubescens	Paratype	F18349	21254	✓		97% orchid root tip OH	unknown	*Urentes*	KF810123
R. variicolor	Isotype	F9513	21259	✓		99% Uncultured Eur	*Amoeninae*	*Amoeninae*	KF810124
R. subcremeiceps	Isotype	F38920	21230	✓		98% *R.* sp. VT	unknown	*Chamaeleontinae*	KF810125
R. alachuana	Isotype	F9510	21221	✓		99% orchid root tip MEX	*Amoeninae*	*Amoeninae*	KF810126
R. albimarginata	Isotype	F19447	21225		✓	99% *R. atropurpurea* TN	unknown	subsect. *Russula*	KF810127
R. levyana	Isotype	F15859	21235	✓		99% *R. xerampelina* BC	*Xerampelinae*	*Xerampelinae*	KF810128
R. vinosirosea	Isotype	F18677	21261	✓		94% *R.* sp. BC	unknown	uncertain	KF810129
R. mutabilis	Isotype	F17943	21237	✓		98% orchid root tip THA	*Subvelatae*	*Ingratae*	KF810130
R. fragiloides	Isotype	F18001	21232	✓		90% *R. raoultii* Eur	subsect. *Russula*	subsect. *Russula*	KF810131
R. australirosea	Isotype	F38859	21228	✓		92% *R.* sp. CA	*Lilaceinae*	uncertain	KF810132
R. emeticiformis	Isotype	F9535	21231	✓		96% *R. atropurpurea* TN	subsect. *Russula*	subsect. *Russula*	KF810133
R. pinophila	Isotype	F17982	21240	✓		96% *R. xerampelina* BC	unknown	*Xerampelinae*	KF810134
R. subsulphurea	Paratype	F18743	21255	✓	✓	100% environ NC	unknown	uncertain	KF810135
R. subrubescens	Isotype	F18339	21253	✓	✓	99% Uncultured MEX	unknown	*Urentes*	KF810136
R. pervirginea Murrill	Isotype	F17266	21238			FAILED			
R. subflava Murrill	Isotype	F16256	21249			FAILED			
R. venusta Murrill	Isotype	F17836	21260			FAILED			
R. subglauca Murrill	Isotype	F17781	21250			FAILED			
R. alutaceiformis Murrill	Isotype	F17703	21226			FAILED			
R. testaceiceps Murrill	Isotype	F15916	21257			FAILED			
R. subobscura Murrill	Isotype	F18301	21251			FAILED			
R. patriotica Murrill	Isotype	F18066	21258			FAILED			
R. subpusilla Murrill	Isotype	F9512	21252			FAILED			
R. mutabilis	none	DPL10654		✓	✓	N/A	*Subvelatae*	*Ingratae*	KF810137
R. cf. *aquosa* Leclair	none	BPL271		✓	✓	N/A	subsect. *Russula*		KF810138
R. vinacea	none	BPL257		✓	✓	N/A	subsect. *Russula*		KF810139

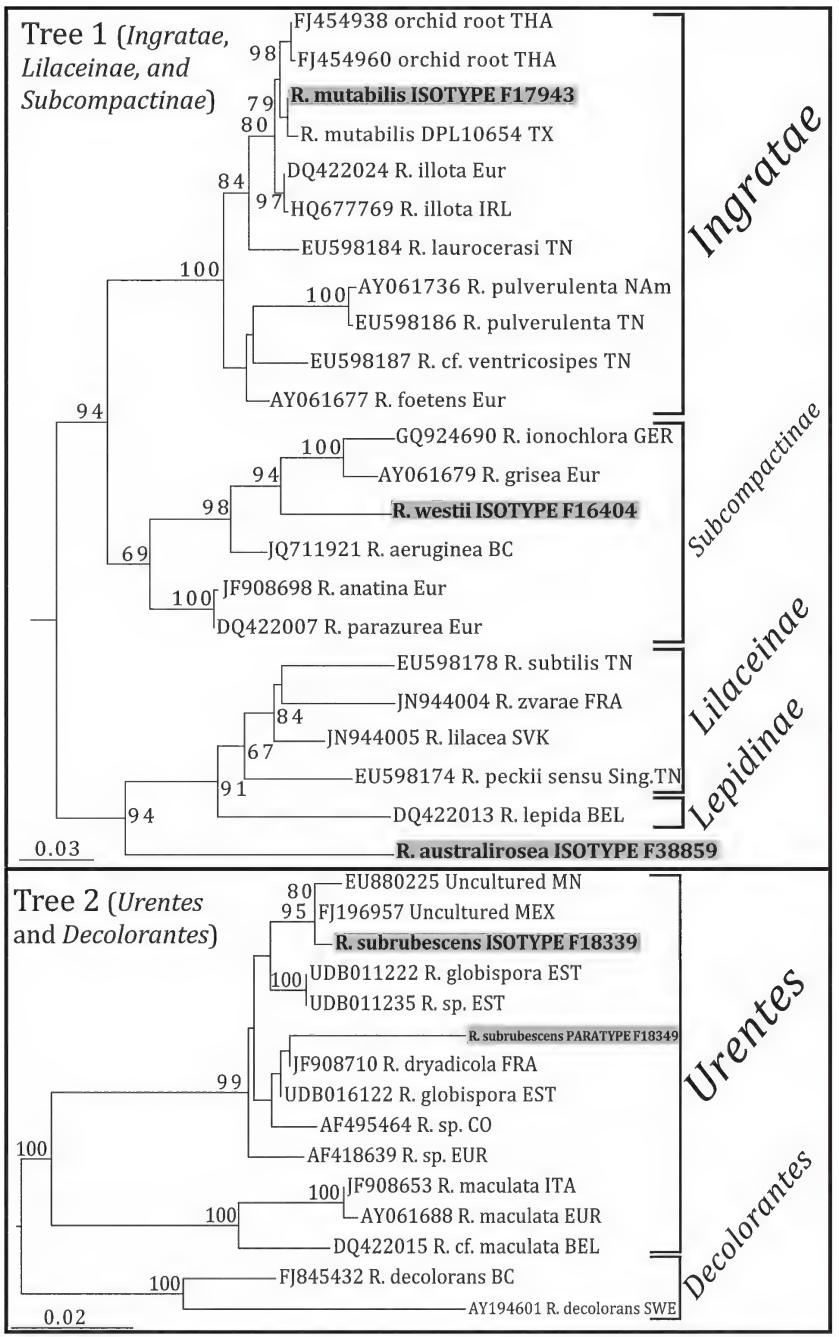
[1]Type collections accessioned at TENN not sampled (out on loan) were: *Russula albiduliformis* Murrill, *R. heterosporoides* Murrill, *R. lutescentifolia* Murrill, *R. pseudofoetens* Murrill, *R. rooseveltiana* Murrill, *R. subalbidula* Murrill, *R. subbrunneipes* Murrill, and *R. subgraminicolor* Murrill.

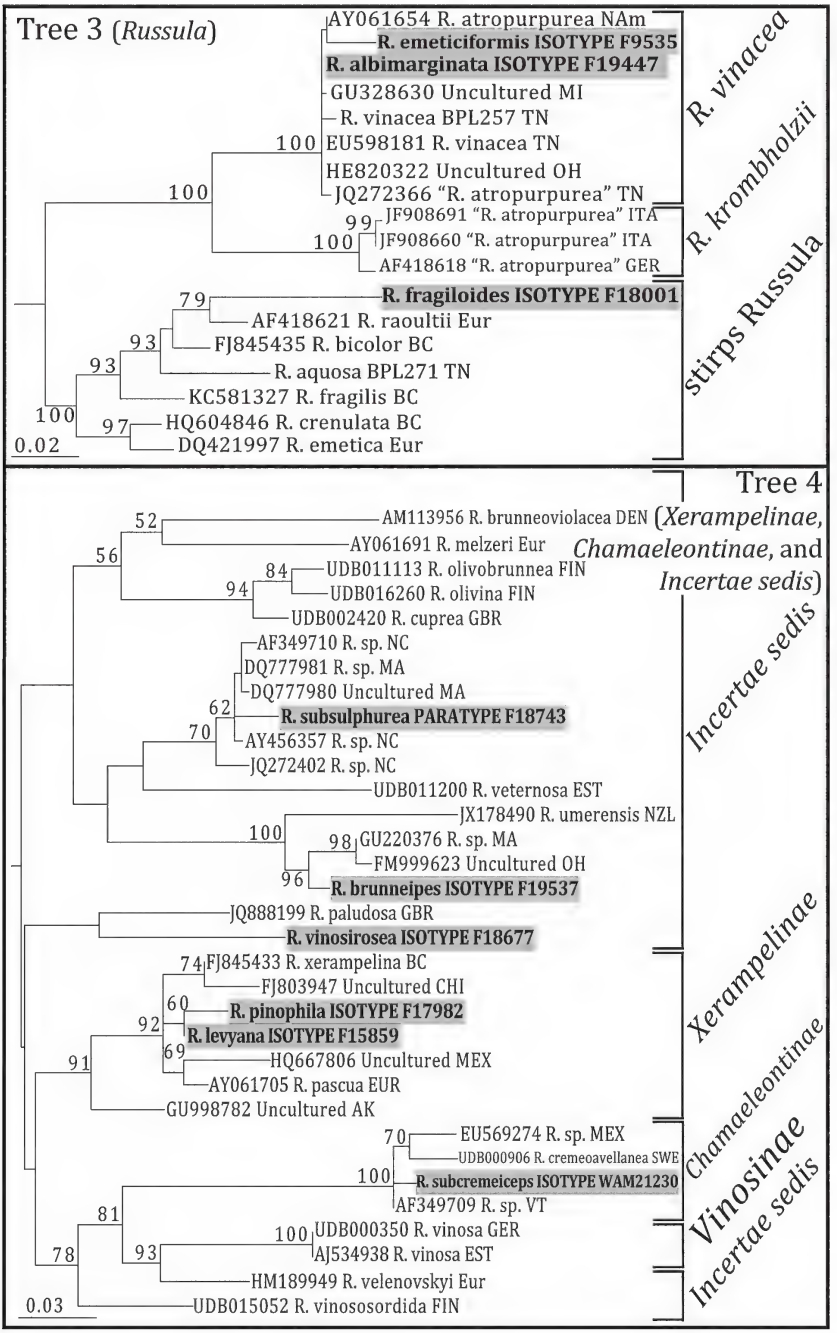
Initially, products were screened using the primer pair ITS1F-ITS4 (Gardes & Bruns 1993, White et al. 1990). PCR amplification protocols and controls used the requisite reagents of sterile water, 5× GoTaq buffer (Promega, Madison, Wisconsin), GoTaq, and 10 mM solution of dNTPs (Invitrogen Corp., Carlsbad, California). The DNA concentration in master mix solution was increased from 1:24 to 4:21 for improved PCR product concentration, and the samples were run using an ITS protocol on a Bio-Rad C1000 thermal cycler (Bio-Rad, Hercules, California). PCR product quality was visualized using gel electrophoresis on a 1% agarose gel prepared with ethidium bromide and then transilluminated using UV light. Specimens for which no banding was present were re-screened using the primer pairs ITS1F-ITS2 and 5.8SR-ITS4. Specimens for which a band was produced were cleaned with a QIAquick PCR purification kit (QIAGEN, Valencia, California). Sequence reactions were prepared and purified following Birkebak et al. (2013). Purified samples were sent to the Molecular Biology Resource Facility at U.T. to be sequenced using a 3730 DNA analyzer (Applied Biosystems, Grand Island, NY).

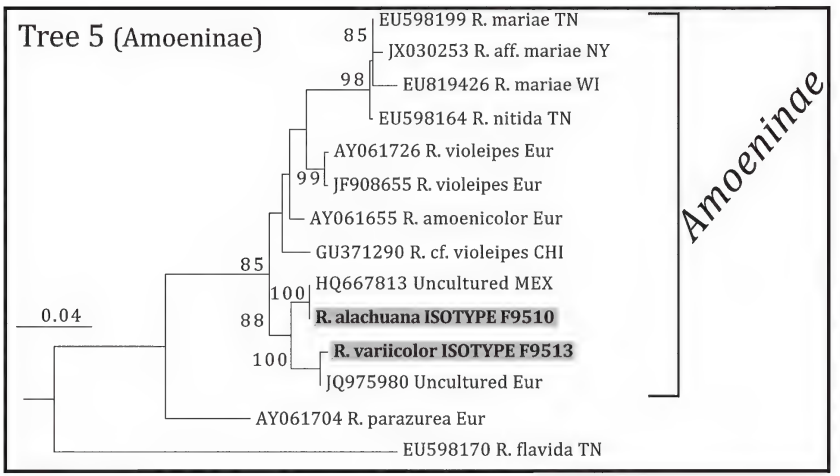
Sequences were assembled using Sequencher 4.9 (Gene Codes Corp., Ann Arbor, Michigan). For types where ITS1 and ITS2 sequences were produced separately, a bridge of repeated N's was used to form a complete ITS sequence. Using the BLAST program (Altschul et al. 1990) sequences were queried against the GenBank database (NCBI, Bethesda, Maryland) and visualized using the distance tree of results. Sequences that blasted with 98% similarity or higher were selected for phylogenetic analysis as candidate conspecifics or closely related taxa as well as identified sequences with at least 90% similarity for placement in an infrageneric group. Additional vouchered sequences of nomenclatural type species or representatives of infrageneric groups based on morphological synapomorphies were selected for testing infrageneric hypotheses. Alignments were formed using MAFFT version 7 (Katoh & Standley 2013) using default options and then adjusted manually in MacClade 4.08 (Maddison & Maddison 2005). Phylip file formats were created in Seaview 4.4.2 (Gouy et al. 2010). Phylogenies were reconstructed using maximum likelihood (ML) in raxmlGUI 1.2 (Silvestro & Michalak 2012, Stamatakis et al. 2008) with 1000 bootstrap replicates. Bootstrap values 70% and above are considered good support for clades. State/provincial abbreviations for the U.S. and Canada are according to national postal codes and country abbreviations use the three-letter ISO code (International Organization for Standardization, Geneva, Switzerland). All sequences are deposited in GenBank.

Molecular results

Sequence data were recovered from sixteen of twenty-five type specimens (64% screened) with collection dates ranging from 1932 to 1944 (Table 1). Only *R. westii* Murrill (KF810121; 4% screened) was recovered as a full ITS sequence using the ITS1F-ITS4 primer pair. When the spacer regions were amplified separately, the ITS1 region was successfully amplified in fourteen of fifteen instances (93%) using the ITS1F-ITS2 primer pair. The ITS2 region was successfully amplified for three out of fifteen types (20%) using the 5.8SR-ITS4 primer pair. Both ITS1 and ITS2 were produced for *R. subrubescens* Murrill


Tree 1 (*Ingratae, Lilaceinae, and Subcompactinae*)
FJ454938 orchid root THA
FJ454960 orchid root THA
R. mutabilis ISOTYPE F17943
R. mutabilis DPL10654 TX
DQ422024 R. illota Eur
HQ677769 R. illota IRL
EU598184 R. laurocerasi TN
AY061736 R. pulverulenta NAm
EU598186 R. pulverulenta TN
EU598187 R. cf. ventricosipes TN
AY061677 R. foetens Eur
GQ924690 R. ionochlora GER
AY061679 R. grisea Eur
R. westii ISOTYPE F16404
JQ711921 R. aeruginea BC
JF908698 R. anatina Eur
DQ422007 R. parazurea Eur
EU598178 R. subtilis TN
JN944004 R. zvarae FRA
JN944005 R. lilacea SVK
EU598174 R. peckii sensu Sing.TN
DQ422013 R. lepida BEL
R. australirosea ISOTYPE F38859
0.03
Ingratae
Subcompactinae
Lilaceinae
Lepidinae
Tree 2 (*Urentes* and *Decolorantes*)
EU880225 Uncultured MN
FJ196957 Uncultured MEX
R. subrubescens ISOTYPE F18339
UDB011222 R. globispora EST
UDB011235 R. sp. EST
R. subrubescens PARATYPE F18349
JF908710 R. dryadicola FRA
UDB016122 R. globispora EST
AF495464 R. sp. CO
AF418639 R. sp. EUR
JF908653 R. maculata ITA
AY061688 R. maculata EUR
DQ422015 R. cf. maculata BEL
FJ845432 R. decolorans BC
AY194601 R. decolorans SWE
0.02
Urentes
Decolorantes


Tree 3 (Russula)
AY061654 R. atropurpurea NAm
R. emeticiformis ISOTYPE F9535
R. albimarginata ISOTYPE F19447
GU328630 Uncultured MI
R. vinacea BPL257 TN
EU598181 R. vinacea TN
HE820322 Uncultured OH
JQ272366 "R. atropurpurea" TN
JF908691 "R. atropurpurea" ITA
JF908660 "R. atropurpurea" ITA
AF418618 "R. atropurpurea" GER
R. fragiloides ISOTYPE F18001
AF418621 R. raoultii Eur
FJ845435 R. bicolor BC
R. aquosa BPL271 TN
KC581327 R. fragilis BC
HQ604846 R. crenulata BC
DQ421997 R. emetica Eur
0.02
R. vinacea
R. krombholzii
stirps Russula
Tree 4
(Xerampelinae, Chamaeleontinae, and Incertae sedis)
AM113956 R. brunneoviolacea DEN
AY061691 R. melzeri Eur
UDB011113 R. olivobrunnea FIN
UDB016260 R. olivina FIN
UDB002420 R. cuprea GBR
AF349710 R. sp. NC
DQ777981 R. sp. MA
DQ777980 Uncultured MA
R. subsulphurea PARATYPE F18743
AY456357 R. sp. NC
JQ272402 R. sp. NC
UDB011200 R. veternosa EST
JX178490 R. umerensis NZL
GU220376 R. sp. MA
FM999623 Uncultured OH
R. brunneipes ISOTYPE F19537
JQ888199 R. paludosa GBR
R. vinosirosea ISOTYPE F18677
FJ845433 R. xerampelina BC
FJ803947 Uncultured CHI
R. pinophila ISOTYPE F17982
R. levyana ISOTYPE F15859
HQ667806 Uncultured MEX
AY061705 R. pascua EUR
GU998782 Uncultured AK
EU569274 R. sp. MEX
UDB000906 R. cremeoavellanea SWE
R. subcremeiceps ISOTYPE WAM21230
AF349709 R. sp. VT
UDB000350 R. vinosa GER
AJ534938 R. vinosa EST
HM189949 R. velenovskyi Eur
UDB015052 R. vinososordida FIN
0.03
Incertae sedis
Xerampelinae
Chamaeleontinae
Vinosinae
Incertae sedis



FIGURES 1–3. Unrooted phylogenies derived from maximum likelihood analysis of complete or partial ITS rDNA sequences of types of *Russula* taxa described by W.A. Murrill with included publicly accessioned sequences for phylogenetic classification. Type sequences are highlighted and bolded. Publicly accessioned sequences are included if they share a 98% sequence similarity to a type or are identified to species and within 90% sequence similarity.

(KF810136) and *R. subsulphurea* Murrill (KF810135). Five phylogenetic trees were constructed by utilizing nomenclatural type species or representatives of infrageneric groups based on morphological synapomorphies to test proposed infrageneric classifications and synonymizations of Murrill's taxa. Monophyletic clades with high bootstrap support that include type species or representatives of known infrageneric classification were used to define infrageneric groups; however, relationships between these clades should not be considered significant due to the lack of gene and taxon sampling.

To test whether the ITS1 region can consistently and accurately separate species in *Russula,* I examined collection FJ845433 from British Columbia, Canada, which shares a 99% sequence similarity with 309/310 sites of the ITS1 region from the isotype of *R. levyana* Murrill. A disjunct or continuous range of over 4000 km across continental North America for one *Russula* species would be surprising, especially considering the significant host, habitat, and climate differences. Although both specimens certainly belong in *R.* subsect. *Xerampelinae* Singer, in the pileus of the Canadian collection, I found large spores, predominantly olive green tones, and no long, attenuated hyphal terminations in the suprapellis — features inconsistent with modern examinations of the type and recent collections of *R. levyana* and which support separation based on a morphological species concept (Adamčík & Buyck 2010).

Tree 1 (*Ingratae*, *Lilaceinae*, and *Subcompactinae*) **FIGURE 1**

The isotype of *R. mutabilis* Murrill shares a 100% sequence similarity with a recent specimen collected in Texas determined as *R. mutabilis* (KF810137). The species is in a well-supported clade that includes sequences identified as *R. illota* Romagn. and environmental sequences of an orchid associate from Thailand. This suggests that *R. mutabilis* is in *R.* sect. *Ingratae* Quél., but its placement in *R.* subsect. *Foetentinae* Melzer & Zvára or *R.* subsect. *Subvelatae* (Singer) Singer, represented by sequences labeled as *R. pulverulenta* Peck, cannot be resolved. *Russula pulverulenta* sequences were chosen because no sequences of *R. subvelata* Singer are available, and *R. pulverulenta* is a commonly collected species with the floccose pilear patches characteristic of *R.* subsect. *Subvelatae*. *Russula mutabilis* and *R. illota* share a benzaldehyde odor (characteristic of *R. fragrantissima* Romagn.), acrid taste, and a staining context, although Singer (1958), who considered its staining an artifact of the drying process, placed *R. mutabilis* in *R.* subsect. *Subvelatae* based on its velar remnants that showed a positive red reaction to potassium hydroxide. A similar species that exhibits the same extreme red staining on both the pileus and stipe is *R. ventricosipes* Peck, which may be a close relative separated by host preference (Adamčík et al. 2013).

The isotype of *R. westii* was recovered in a well-supported clade with members of *R.* subsect. *Subcompactinae* Singer, separate from a clade containing members of *R.* subsect. *Lilaceinae* Melzer & Zvára, including the type species. Morphological characters of *R. westii* are not inconsistent with placement in *R.* subsect. *Subcompactinae*, which includes a cream spore print, mild taste, and verrucose spores that lack an amyloid suprahilar spot (Murrill 1941). Singer (1958) noted an absence of pileocystidia and the presence of "none or few" primordial hyphae, but a preliminary morphological examination of the isotype by Adamčík revealed conspicuous pileocystidia. To determine whether the type collection was mixed and the TENN material represents a different species, the isotype was compared with a more detailed study of the FLAS holotype and confirmed as conspecific (Adamčík pers. comm.). Singer (1958) noted that *R. westii* was morphologically and ecologically very similar to *R. cremea* (Murrill) Singer and differed only in spore morphology.

Contrasting with Singer's (1986) classification, the phylogeny places the isotype of *R. australirosea* Murrill outside the clade representing *R.* subsect. *Lilaceinae,* united by the presence of primordial hyphae, a brightly colored pileus, and a pale spore print; unfortunately it is not possible at this time to place *R. australirosea* in another group. In his type study, Singer (1958) characterized *R. australirosea* with a completely mild taste and lacking dermatocystidia, characters consistent with *R.* subsect. *Lilaceinae* but described the spore print color as C to D, darker than expected for *R.* subsect. *Lilaceinae*. Singer (1958)

proposed that *R. australirosea* might be conspecific with *R. vinosirosea* Murrill, but we can reject this based on its phylogenetic placement.

Tree 2 (*Urentes* and *Decolorantes*) **Figure 1**

The *R. subrubescens* isotype and paratype form a clade with closely related sequences, but the sequences share only a 94% ITS similarity and are separated in two distinct clades with strong support. The BLAST results support the *R. subrubescens* type as closely related to *R. globispora* (J. Blum) Bon while placing the paratype in a clade with a sequence labeled "*R. dryadicola*" and another *R. globispora* sequence. Singer (1986) did not treat either of these taxa specifically, although he might have included them as varieties of *R. maculata* Quél., united by acrid taste, ochre spore print, absence of iodoform odor, and numerous dermatocystidia. Therefore, both entities should be included in *R.* subsect. *Urentes* Maire, not in *R.* sect. *Decolorantes* (Maire) Singer as represented by the type species *R. decolorans* (Fr.) Fr. Metadata associated with environmental sequences suggest that *R. subrubescens* is distributed throughout temperate North America as an associate of *Quercus*.

Tree 3 (*Russula*) **Figure 2**

Russula albimarginata Murrill and *R. emeticiformis* Murrill are both recovered in a clade with *R. vinacea* Burl., which is separate from the European representatives of *R. krombholzii* Shaffer[1] labeled "*R. atropurpurea*". Shaffer (1970) noted that *R. vinacea* reportedly has a less acrid taste and stronger yellowing flesh than *R. krombholzii*. The name *R. albimarginata* likely refers to the pallid margin described by Murrill (1945a) and observed in fresh collections (particularly in young fruitbodies) of *R. vinacea*. Singer's (1958) proposed placement of *R. emeticiformis* in *R.* subsect. *Russula* as a synonym of a nominal subspecies of *R. emetica* (Schaeff.) Pers. is not supported, as *R. emetica* is distantly related in *Russula* stirps Emetica. Singer's later (1975) placement of *R. emetica* subsp. *lacustris* Singer in *Russula* stirps Atropurpurea does, however, correspond with the current phylogenetic placement of *R. emeticiformis*. Both *R. albimarginata* and *R. emeticiformis* should be considered later taxonomic synonyms or closely related species to *R. vinacea*.

The isotype of *R. fragiloides* Murrill falls in a well-supported clade with members of *R.* subsect. *Russula* stirps Russula. Singer (1958) suggested that *R. fragiloides* is synonymous with *R. emetica* subsp. *emeticella* (Singer) Singer [≡ *R. emeticella* (Singer) Romagn.], but a recent revision by Hampe et al. (2013)

[1]Singer (1986: 824) used a heterotypic synonym, *R. bresadolae* Schulzer 1886, for the illegitimate later homonym *R. atropurpurea* (Krombh.) Britzelm. 1893, non Peck 1888. However the correct name for this taxon is *Russula krombholzii*, which was published as a replacement name (nom. nov.) based on the legitimate synonym *Agaricus atropurpureus* Krombh. 1845, and which therefore has priority over *R. bresadolae*.

found this taxon's position to be uncertain due to confusion over assigning a lectotype.

Tree 4 (*Xerampelinae* and Incertae sedis) **FIGURE 2**

Murrill (1945a,b) described *R. levyana* and *R. pinophila* Murrill with unchanging context, no odor, and — in the case of *R. pinophila* — a white gill color, features that would generally exclude these taxa from *R.* subsect. *Xerampelinae,* although he regarded *R. levyana* as related to *R. xerampelina* (Schaeff.) Fr. Adamčík & Buyck (2010), who evaluated the type and recent collections of *R. levyana,* noted these inconsistencies but explained that some American representatives of this group are known for having weak odors, especially in young fruitbodies. Younger fruitbodies might also explain gill whiteness, as *R.* subsect. *Xerampelinae* is characterized by ochraceous spore prints that make mature gills appear yellow. Phylogenetically, *R. levyana* and *R. pinophila* are in a well-supported clade with representatives of *R.* subsect. *Xerampelinae*. These two species share a 98% ITS sequence similarity and may represent the same species, a conclusion supported by their association with pine (Adamčík 2010). If so, *R. pinophila* should be regarded as a later taxonomic synonym of *R. levyana*. However, Buyck (pers. comm.) has suggested that species delimitation in this particular group should use a sequence similarity cutoff greater than 98%.

The isotype of *R. subcremeiceps* Murrill is closely related to *R. cremeoavellanea* Singer, which Singer (1986) placed in *R.* subsect. *Chamaeleontinae* Singer based on its eventual chocolate brown reaction to phenol, deep ochraceous spore print, and mild taste. Murrill (1946) did not test specimens for their phenol reaction, but described *R. subcremeiceps* as having a mild taste and pale yellow spore print. *Russula subcremeiceps* is somewhat allied with two other groups: *R.* subsect. *Vinosinae* Singer represented by *R. vinosa* Lindblad and *R.* subsect. *Integrae* Maire represented by *R. velenovskyi* Melzer & Zvára (Singer 1986). Both *R. cremeoavellanea* and *R. vinosa* are species that discolor, but Murrill (1946) described *R. subcremeiceps* as having unchanging flesh. He also noted that *R. subcremeiceps* closely resembled *R. albidicremea* Murrill but differed by having closer unforked gills and rounder spores. *Russula subcremeiceps* is a species widely distributed across eastern North America ranging from the northeast U.S. to Mexico.

Morphologically, *R. vinosirosea* is distinguished by its rose color with pale vinose tint and subequal stipe that is shorter than pileus width (Murrill 1943), two characters consistent with *R.* subsect. *Integrae* sensu Singer, of which *R. paludosa* Britzelm. is the type species. Although the isotype of *R. vinosirosea* shares 92% identity with *R. paludosa,* as well as *R. olivobrunnea* Ruots. & Vauras, *R. olivina* Ruots. & Vauras, and *R. veternosa* Fr., it cannot be confidently assigned to a taxonomic group due to low phylogenetic resolution.

Russula subsulphurea is distinguished by its large (9–10 cm) solitary habit, pallid to yellow-tinted to slightly rosy pileus, and white spore print (Murrill 1945b). The phylogenetic placement of the paratype specimen is unknown, but multiple environmental studies have frequently sampled *R. subsulphurea* or a closely related species in eastern North America.

The isotype of *R. brunneipes* Murrill is closely related to *R. umerensis* McNabb from New Zealand. These two species share many characters such as presence of dermatocystidia, vinaceous pileus color, a 7.5–10 µm spore size, and spores ornamented by mostly isolated echinulate 0.5–1 µm tall spines (Hesler 1960, McNabb 1973, Murrill 1945a). The *R. brunneipes* isotype shares a 92% identity with *R. cuprea* (Krombh.) J.E. Lange, *R. vinososordida* Ruots. & Vauras, and *R. vinosa*, but taxonomic placement is impossible at this time.

Tree 5 (*Amoeninae*) **FIGURE 3**

Russula alachuana Murrill and *R. variicolor* Murrill form a well-supported clade with other members of *R.* subsect. *Amoeninae* Singer. No reliable sequence of *R. amoena* Quél. was available, but all included taxa are united by having cheilocystidia without contents, no dermatocystidia, and a pale yellow to yellow spore print (Singer 1986). Both species form well-supported subclades separated from a well-sampled clade of specimens identified as *R. mariae* Peck, indicating that these are discrete phylogenetic species. Morphologically, *R. alachuana* is distinct by its large size, velvety pileus, and pulverulent stipe with rosy hue (Murrill 1938). An environmental sample of *R. alachuana* or closely related species has been sampled from Mexico. Murrill (1942) distinguished *R. variicolor,* potentially a trans-continental species sharing a close genetic affinity (99% similarity) to a *Pinus pinaster*-associated root tip sample from Europe, by its many-colored pileus that includes greenish hues and a completely white stipe.

Discussion

A molecular sequence of type material is an invaluable tool for modern systematics because it gives the taxonomist somewhat more objective criteria for naming phylogenetic groups and delimiting new species. The ITS region has been suggested as a barcoding region for ectomycorrhizal fungi due to the ease of its amplification and its ability to separate intraspecific from interspecific taxa (Schoch et al. 2012). My study illustrates a major hurdle in the revival of North American *Russula* taxonomy, which is that Murrill and his predecessors, G.S. Burlingham and C.H. Peck, described most *Russula* species in the 1910's–1940's. This means that North American type material is at the cusp of what can be successfully sequenced, and given the large number of species described and even more yet to be described, sequences of these taxa can greatly aid the revival of North American *Russula* systematics.

In this study I was able to produce ITS data from type collections designated by W.A. Murrill between 1938 and 1948 with a 66% success rate for collections accessioned at TENN. However, only the ITS1 region was successfully sequenced for a majority of Murrill's type material, and there are questions as to the value of using only ITS1 or ITS2 as a species barcode to separate closely related species. Either ITS region has been considered adequate for delimiting species in some groups of fungi, but this might not work as well in speciose groups, where species delimitation is performed at a fine scale with closely related taxa (Bengtsson-Palme et al. 2013).

Comparison of the isotype collection of *R. levyana* with FJ845433 (the specimen that shares a 99% sequence similarity) highlights the problem of using morphological or phylogenetic approaches separately in taxonomy. Either we must accept morphological variation in widespread species that may have been over-split and over-described or regard the ITS1 region as inadequate for separating closely related *Russula* species. To overcome these limitations, I suggest combining morphological recognition with molecular data to designate recently collected material as epitypes for historical North American *Russula* species. A good example can be found in *R. mutabilis*, where a partial sequence of the type shows a 100% sequence similarity with a recent collection from Texas that also agrees with the morphological diagnosis of the type.

Although partial sequence data may not allow for complete confidence in species delimitation, we are able to use such data to infer placement in clades, thereby supporting or refuting past classifications based on morphology. From the eight sampled taxa that have never before been placed in a group, I can now confidently place four: *R. albimarginata* in *R.* subsect. *Russula*, *R. pinophila* in *R.* subsect. *Xerampelinae*, *R. subcremeiceps* in *R.* subsect. *Chamaeleontinae*, and *R. subrubescens* in *R.* subsect. *Urentes* (Table 1). The results indicate a different or refined placement of *R. westii* in *R.* subsect. *Subcompactinae*, and we now have a more refined placement of *R. fragiloides*, *R. alachuana*, *R. variicolor,* and *R. emeticiformis* within their subsections (Table 1). Two taxa, *R. albimarginata* and *R. emeticiformis*, are proposed as possible synonyms with *R. vinacea*, and *R. pinophila* may be considered a possible synonym of *R. levyana*. Knowledge of group placement will aid in future systematic revisions, and those species not yet referable to an identifiable clade may be placed with additional molecular sampling and reliably identified sequences accessioned in GenBank and curated databases like UNITE.

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Molecular phylogeny of placodioid lichen-forming fungi reveal a new genus, *Sedelnikovaea*

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Abstract — A molecular phylogeny based on ITS1/ITS2 and mtSSU DNA sequences is presented for lichen-forming fungi in *Protoparmeliopsis* and its distant relative *Sedelnikovaea* gen. nov. The positions of the two genera in the phylogenetic tree of the families *Lecanoraceae* and *Lecideaceae* are discussed. The new combination *Sedelnikovaea baicalensis* (≡ *Lecanora baicalensis*) is proposed. The unique *Sedelnikovaea*-type ascus is described and illustrated. *Sedelnikovaea baicalensis* and *Zwackhiomyces zarei* are for the first time recorded from China.

Key words — *Biatora*, *Rhizoplaca*, *Protoparmelia*, *Candelariella*, *Steinia*, *Pycnora*

Introduction

The lichen-forming fungal genus *Protoparmeliopsis* comprises a total of ca. 44 species (Santesson 2004; Kondratyuk 2010; Kondratyuk et al. 2012, 2013 b). After Arup & Grube (2000) provided the first molecular data for *Protoparmeliopsis*, five new species and 42 new combinations have been proposed in the genus (Kondratyuk 2010; Kondratyuk et al. 2012, 2013b; Santesson 2004).

Protoparmeliopsis is not currently classified in the *Lecanoraceae* (Lumbsch & Huhndorf 2007, 2010) due, in part, to lack of molecular data and unresolved relationships within *Lecanora* sensu lato. Sequences from only three species — *P. achariana* (A.L. Sm.) Moberg & R. Sant., *P. macrocyclos* (H. Magn.) Moberg

& R. Sant., and *P. muralis* (Schreb.) M. Choisy — are available in GenBank. Several species recently described in *Protoparmeliopsis* (or recently combined in the genus based on morphology; Kondratyuk 2010, Kondratyuk et al. 2012, 2013 b) are here included in a molecular analysis.

Material & methods

Specimens were prepared using standard microscopical techniques: specimens were hand-sectioned under Nikon SMZ645 dissecting microscope, examined under Nikon E200 and Olympus BX51 microscope, and photographed using the Olympus DP-Soft photo program.

Table 1. Specimens included in the phylogenetic analysis

Taxon name	Country / voucher	ITS1/ITS2	12S SSU mt DNA
Adelolecia pilati	Norway?, Ekman 3373 (BG)		AY567713
	Norway?, Ekman 3373 (BG)		AY300874
Aspicilia caesiocinerea	AFTOL-ID 653		DQ986892
Aspicilia cinerea	AFTOL-ID 647		DQ986890
	Sweden	EU057899	
Boreoplaca ultrafrigida	China		HQ026247
	AFTOL-ID 1702		DQ986813
	Russia		AY853312
Circinaria calcarea	Sweden	EU057898	
	Sweden		AY853310
	Austria?/ Wilfling (GZU)	AF332108	
	AFTOL-ID 1358		DQ986876
Circinaria desertorum	USA	EU057905	
	Russia		HM060689
Circinaria hispida	Iran	HQ389197	
	SK A12, Ukraine, Kharkiv oblast, Dvorychansky district, 'Korobchyno' zakaznik, 19.vi.2013 M. Kryvokhyzhaya (KW-L)		**KP059052**
	SK A15, Ukraine, Kharkiv oblast, Dvorychansky district, 'Korobchyno' zakaznik, 19.vi.2013 M. Kryvokhyzhaya 2 (KW-L)		**KP059053**
	Turkey		HM060722
Farnoldia jurana	Austria		GU074511
Hypocenomyce scalaris	AFTOL-ID 1025	HQ650632	
	Sweden		AY853325
	United Kingdom	FR799187	
	Sweden		AY853326
	AFTOL-ID 687		DQ912274
Japewia tornoensis	Canada	EF495163	
	?/ Printzen s. n.	HQ650656	
	Canada		HQ660559
Lecanora achroa	Thailand	JN943715	
	Australia	JN943719	
Lecanora albescens	Poland?/ Kubiak Jan. 2009 (KRAM)	JQ993727	
Lecanora allophana	Norway?, Ekman 3434 (BG)		AY567710
Lecanora argentata	Thailand	JQ782704	

Taxon name	Country / voucher	ITS1/ITS2	12S SSU mt DNA
Lecanora bicincta	Austria?/ U. Trinkaus 102 (GZU)	AY541264	
Lecanora caesiorubella	Australia		JQ782672
Lecanora campestris	Sweden?/ U225	AF159930	
	Sweden?/ U514		DQ787362
Lecanora carpinea	Sweden?/ U507		DQ787364
	Austria?/ J. Pruegger 61232 (GZU)	AY541247	
Lecanora crenulata	Poland?/ Sliwa 4087 (KRAM)	JQ993725	
Lecanora contractula	AFTOL-ID 877	HQ650604	
	AFTOL-ID 877		DQ986898
Lecanora dispersa	Hungary?/Lőkös Jan. 2009 (KRAM)	JQ993733	
Lecanora dispersoareolata	Austria?/ M124	AF070016	
Lecanora farinacea	Austria?/ U. Trinkaus 115 (GZU)	AY541261	
	Australia	JN943725	JQ782672
	Australia		JQ782670
Lecanora flavopallida	Australia	JN943723	JQ782674
	Australia		JQ782673
Lecanora fuscescens	Hauck s. n.	HQ650652	
Lecanora gangaleoides	USA		JQ782676
Lecanora glabrata	Sweden?/ U444		DQ787360
Lecanora hagenii	Hungary?/ Lőkös Jan. 2009 (KRAM)	JQ993735	
Lecanora hybocarpa	?/ Lumbsch s.n. (F)		EF105417
Lecanora intricata	Austria ?	AY398703	
Lecanora intumescens	Norway?, Ekman 3162		AY567715
Lecanora kenyana	Kenya	JQ900618	
Lecanora novomexicana	USA	HM577255	
Lecanora paramerae	?/ Lumbsch s.n. (F)		EF105418
Lecanora perpruinosa	Sweden?/ U176	AF070025	
Lecanora polytropa	Antarctica, Hur ANT050752	DQ534470	
Lecanora reuterii	Poland?, Sliwa 4113 (KRAM)	JQ993741	
Lecanora rupicola	Spain	DQ451670	
Lecanora semipallida	Poland?/ Sliwa 4102 (KRAM)	JQ993755	
Lecanora subcarpinea	Slovenia	DQ451657	
Lecanora sulphurea	Sweden?/ U508		DQ787356
Lecanora swartzii subsp. *caulescens*	Austria?/ M. Grube s.n. (GZU)	AY541272	
Lecanora swartzii	Austria	DQ451655	
Lecanora tropica	Kenya	JN943718	
	Thailand	JN943720	JQ782699
Lecanora valesiaca	Sweden?/ U512		DQ787350
Lecidea fuscoatra	AFTOL-ID 589	HQ650707	
	Sweden, Arup L02723 (Hb. Arup)		HQ660567
	Turkey	HQ605929	
	Turkey	HQ605927	
	Sweden?/ Wedin 6860 (UPS)		AY756401
	AFTOL-ID 589		DQ912275
Lobothallia alphoplaca	USA	JX306737	
	USA	JX306739	
Lobothallia melanaspis	Norway	JF825524	
	Sweden		HM060688
Lobothallia radiosa	Sweden	JF703124	
	Switzerland		DQ780274
Lobothallia recedens	Sweden		HM060724

Taxon name	Country / voucher	ITS1/ITS2	12S SSU mt DNA
Megaspora verrucosa	Austria? /1996, Trinkaus (GZU)	AF332121	
	Austria?/ Hafellner 48544 & Ivanova (GZU)	AF332122	
	Sweden		HM060687
	Iran		JQ797483
Protoparmelia atriseda	USA	KF562190	
Protoparmelia badia	Austria	KF562191	
	Sweden?/ U167	AF070023	
	Australia		AF351179
	Austria	EU075540	
	?/ Lumbsch s.n. (F)		EF105420
Protoparmelia cupreobadia	USA	KF562192	
Protoparmelia isidiata	USA	JF821184	
Protoparmelia montagnei	BCC-Lich 13178	AF101277	
Protoparmelia picea	Norway	KF562194	
Protoparmelia psarophana	BCC-Lich 13180	AF101279	
Protoparmeliopsis achariana	Sweden?/ U155	AF070019	
	Sweden?/ U525		DG787342
Protoparmeliopsis garovaglii	Austria?/ M107	AF189718	
Protoparmeliopsis macrocyclos	Sweden?/ U273	AF159933	
Protoparmeliopsis muralis	SK 765, Romania, 2011 J.-S. Hur (RO11-130) (KoLRI)	**KP059048**	**KP059054**
	Sweden?/ U509		DQ787340
	Poland	HM209239	
	Schmull s. n.	HQ650653	
Protoparmeliopsis zarei	SK 480, Iran, Esfahan Province, Mooteh Wildlife refuge, 50°39′56″E 33°25′34″N, 2510 m alt., 21.VII.2010 B. Zarei-Darki (1111) (KW-L)	**KP059049**	**KP059055**
	SK 481, Iran, Esfahan Province, Mooteh Wildlife refuge, 50°39′56″E 33°25′34″N, 2510 m alt., 21.VII.2010 B. Zarei-Darki (1108) (KW-L)		**KP059056**
Pycnora sorophora	Sweden, Hermansson 7903a (UPS)	FJ959357	AY853338
Pycnora xanthococca	Sweden, Hermansson 11849 (UPS)	AY853388	AY853339
Pyrrhospora quernea	Norway?, Ekman 3019 (BG)	AF517930	AY567712
	Norway?, Ekman 3019 (BG)		AY300908
Pyrrhospora sanguinolenta	Australia, Elix 28835 (F)	EU075548	
Ramboldia brunneocarpa	Australia	EU075542	
Ramboldia stuartii	Australia	EU075549	
Rhizoplaca bullata	Spain		AY464070
Rhizoplaca chrysoleuca	USA	HM577253	
	Sweden?/ U503		DQ787354
	China, Wei, 3, 8/5/2002	AY509799	
Rhizoplaca haydenii	USA	HM577302	
Rhizoplaca idahoensis	USA	HM577297	
Rhizoplaca melanophthalma	Switzerland	JX948232	
	Sweden?/ U519		DQ787352
Rhizoplaca occulta	USA	HM577307	
Rhizoplaca parilis	Chile	JX948227	
Rhizoplaca peltata	China, Wei, 22-c, 2001	AY530887	
	China, Guo, 3557, 8/1/2003	AY509802	
Rhizoplaca polymorpha	USA	JX948194	

Taxon name	Country / voucher	ITS1/ITS2	12S SSU mt DNA
Rhizoplaca porteri	USA	HM577381	
Rhizoplaca shushanii	USA	HM577294	
Rhizoplaca subdiscrepans	China, Wang, 13-2, 8/4/2002	AY509789	
Scoliciosporum chlorococcum	United Kingdom	FR799323	
	Norway?, Ekman 3390 (BG)		AY567768
Scoliciosporum umbrinum	Austria?, A. Wilfling 2873 (GZU)	AY541277	
	Norway?, Ekman 3005 (BG)		AY567719
	Norway?, Ekman 3005 (BG)		AY300911
Sedelnikovaea baicalensis	SK A13, China, Inner Mongolia, 42°01′011″N, 116°17′738″E, 1434 m alt., 8.viii.2009 J.-S. Hur & X.Y. Wang CH090322 KoLRI 010936	**KP059050**	**KP059057**
	SK 776, China, Inner Mongolia, 42°01′011″N, 116°17′738″E, 1434 m alt., 8.viii.2009 J.-S. Hur & X.Y. Wang CH090322 KoLRI 010936	**KP059051**	**KP059058**
	SK A18, China, Inner Mongolia, 43°36′272″N, 116°43′242″E, 1260 m alt., 10.viii.2009 J.-S. Hur & X.Y. Wang CH090352 (KoLRI 010966)		**KP059059**
Steinia geophana	Germany		JN222812
Xanthomendoza mendozae	Chile	EU681351	
	Chile	EU681349	EU680937
	Argentina	EU681350	EU680938
Xanthomendoza kashiwadani	Chile		EU680936
Xylopsora friesii	United Kingdom Sweden	FR799184	 AY853324

Results

We submitted data on *P. muralis* and *P. zarei* S.Y. Kondr. into GenBank for the first time, as ITS rDNA and 12SSU mtDNA sequence analyses confirm both species within *Protoparmeliopsis*. Phylogenetic analysis also shows that another Asian species, *P. baicalensis* (Zahlbr.) S.Y. Kondr., is outside the genus and very distant from *Protoparmeliopsis*. Our anatomical examination of material of *'Protoparmeliopsis' baicalensis* revealed that this taxon is characterized by a unique ascus-type (Pl. 3), which we henceforth refer to as the *Sedelnikovaea*-type. Consequently we propose a new genus, *Sedelnikovaea,* for this species, *S. baicalensis*.

Sedelnikovaea baicalensis and *Zwackhiomyces zarei* S.Y. Kondr. (KoLRI 10971, on *Rhizoplaca chrysoleuca*, see below under specimens examined) are for the first time recorded from China.

Taxonomy & phylogeny

Protoparmeliopsis M. Choisy, Bull. Soc. Bot. France 76: 523 (1929).

Type species — *Lichen muralis* Schreb. [≡ *Protoparmeliopsis muralis*]

Thallus commonly distinctly placodioid. Apothecia lecanorine. Asci of *Lecanora*-type. Ascospores hyaline, simple.

Protoparmeliopsis comprises approximately 44 species. For a detailed description of the genus and its history, see Kondratyuk (2010).

Molecular phylogeny — As a monophyletic group *Protoparmeliopsis* has high bootstrap support based on results from nuclear (ITS1/ITS2) and mitochondrial (12S SSU) DNA sequence analyses that included four *Protoparmeliopsis* species (Figs 1, 2).

The status of such species as *P. macrocyclos* and *P. garovaglii* (Körb.) S.Y. Kondr. still requires further study.

Protoparmeliopsis is not generally accepted (Lumbsch & Huhndorf 2007, 2010), probably because the *Lecanora* s. lat. clade itself has a rather high bootstrap support (see Pl. 2). However, relationships among lineages within *Lecanora* s. lat. remain largely unresolved, and improved sampling is required to circumscribe genera within this group adequately.

A parallel case is the *Dufourea* s.lat. clade in *Xanthorioideae, Teloschistaceae* (Kondratyuk et al. 2014b, and Fedorenko et al. 2009, 2012; Kondratyuk et al. 2013a as *Xanthodactylon* s.lat. clade). However within this clade *Dufourea* Ach. s.str., *Jackelixia* S.Y. Kondr. et al., *Ovealmbornia* S.Y. Kondr. et al., *Langeottia* S.Y. Kondr. et al., and *Xanthokarrooa* S.Y. Kondr. et al. have strong individual bootstrap support values, with each monophyletic branch also characterized by a unique set of morphological, anatomical, and biochemical characters.

Similarly ITS1/ITS2 nrDNA (Pl. 1) and 12S SSU mtDNA (Pl. 2) analyses strongly support *Protoparmeliopsis* within the *Rhizoplaca*/*Protoparmeliopsis* clade. The genus is probably as polyphyletic as *Rhizoplaca* Zopf (Arup & Grube 2000, Arup et al. 2007, Leavitt et al. 2011, 2013), *Protoparmelia* M. Choisy (Lendemer & Lumbsch 2008, Papong et al. 2011, Sing et al. 2013), and *Lecanora* Ach. (Grube & Blaha 2003, Grube et al. 2004, Lumbsch et al. 2012, Sliwa et al. 2012).

Sedelnikovaea S.Y. Kondr., M.H. Jeong & Hur, **gen. nov.**

MycoBank 810705

Differs from *Protoparmeliopsis* in having a unique type of ascus in some respects similar to *Candelariella*- and *Biatora*-type, and by its close molecular similarity to members of the *Lecideaceae*.

Type species — *Lecanora baicalensis* Zahlbr. [≡ *Sedelnikovaea baicalensis*]

Etymology — honouring lichenologist Nellia Vasiljevna Sedelnikova (Novo-sibirsk, Russia) in recognition of her contribution to our knowledge of Asian lichen flora.

Thallus distinctly placodioid, rosette-like, apothecia lecanorine, asci of *Sedelnikovaea*-type, 8-spored; ascospores simple, hyaline.

Taxonomic position — This new genus is proposed for the single species *Sedelnikovaea baicalensis*, morphologically similar but only distantly related to species in the putative genus *Protoparmeliopsis*. Molecular data (see

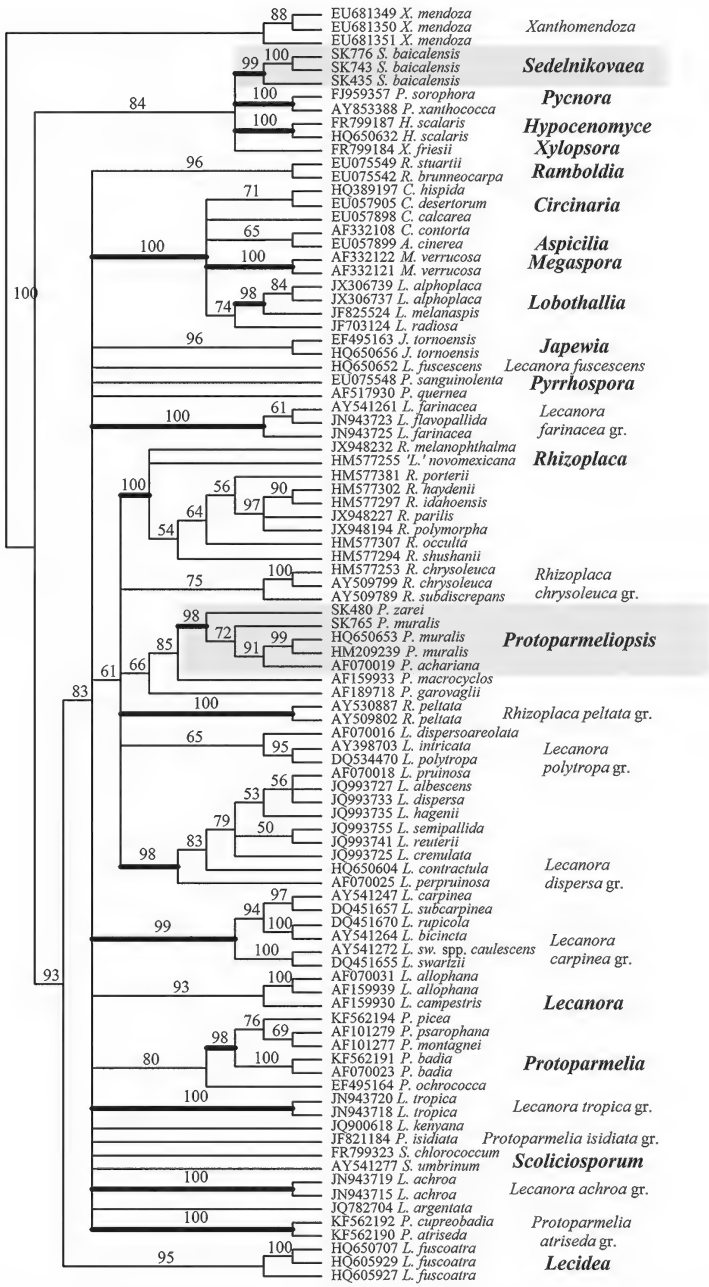

PLATE 1. The phylogenetic tree of *Protoparmeliopsis* and *Sedelnikovaea* and representatives of *Lecanoraceae* and *Lecideaceae*, based on the ITS1/ITS2 gene of nuclear DNA.

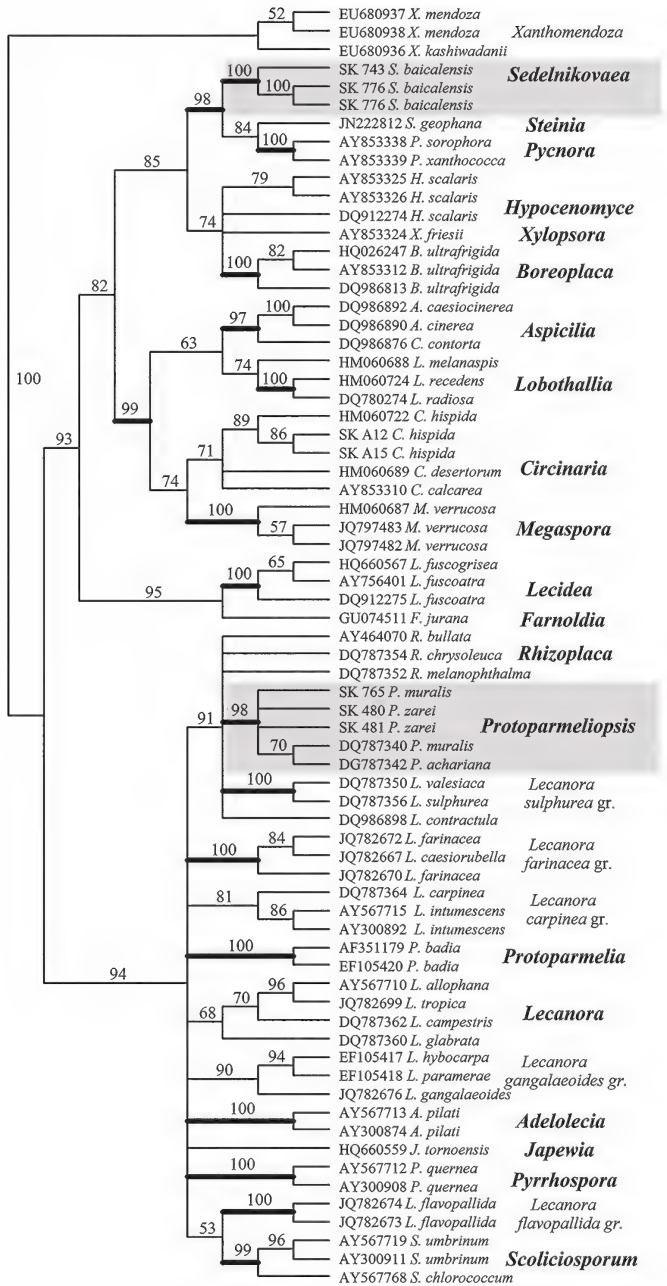

PLATE. 2. The phylogenetic tree of *Protoparmeliopsis* and *Sedelnikovaea* and representatives of *Lecanoraceae* and *Lecideaceae*, based on the 12S SSU gene of mitochondrial DNA.

below) indicate a close relationship with the *Lecideaceae* or *Megasporaceae*. Morphological and anatomical comparisons show important differences from *Protoparmeliopsis* species, primarily a somewhat grey-brownish (instead of greenish) thallus and possession of a unique *Sedelnikovaea*-type ascus, described below.

Sedelnikovaea baicalensis (Zahlbr.) S.Y. Kondr., M.H. Jeong & Hur, **comb. nov.**
MycoBank 810706 Pl. 3–4

≡ *Lecanora baicalensis* Zahlbr., Trav. Sous-Sect. Troitzkossawsk.-Khiakta, Sect. Pays d'Amour, Soc. Imp. Russe de Géogr. 12: 85. 1911 ["1909"].
≡ *Placolecanora baicalensis* (Zahlbr.) Kopach., Nov. Sist. Niz. Rast. 9: 293. 1972.
≡ *Protoparmeliopsis baicalensis* (Zahlbr.) S.Y. Kondr., Ukr. Botan. Zhurn. 96 (6): 876. 2012.

Thallus crustose, rosette-like, up to 1–2.5 cm across, often forming larger aggregations, rather thick, in section to 1 mm thick, densely attached to the substrate, distinctly lobate in peripheral zone and sometimes areolate in the centre, upper surface rough to slightly verruculose, matt to slightly shiny, grayish brown to brownish, yellow-brownish or ochraceous-brown. Lower surface dark brown to blackish-brown. Apothecia up to 1–1.5 mm diam., lecanorine (in section zeorine), mainly rounded, attenuated at the basis, disc shiny at first, subconvex to convex when mature, light-brown to light reddish brown; thalline margin narrow, entire to crenulate, sometimes disappearing in senescence. True exciple up to 30 µm thick in lateral portion and somewhat indistinct in basal portion. Hymenium up to 50–70 µm high, epihymenium 15–20 µm thick, brownish. Subhymenium up to 100 µm thick, underlying an algal layer up to 50–80 µm thick. Paraphyses rather lax. Asci 50–57 × (13–) 15–18(–20) µm. Ascospores ellipsoid, 11-18 × 5-7 µm. Thallus K–, C–, Pd+ slightly reddish.

Specimens examined – **RUSSIA. Chita region (oblast)**, Kyrinsky district, 2 km SW of Chita-Khapcheranga junction, 49°45′21.40″N 112°20′08.60″E, 1184 m alt., shale rocks, 28 July 2008 Yakovchenko (VBGI); Sokhondinskiy biosphere reserve, vicinity of 'Buninda' Forest Station, 49°42′20.46″N 111°21′55.36″E, 1294 m alt., experimental plot 31, Steppe S-facing rocky slope with granitoid stones, 24 July 2008 Yakovchenko (VBGI); Sokhondinskiy biosphere reserve, vicinity of 'Agutsa' Forest Station, 49°39′57.16″N 111°25′39.11″E, 1130 m alt., Steppe S-facing rocky slope with *Pinus sylvestris* in the upper part of the slope near the Station, 02 September 2009 Yakovchenko (VBGI); Sokhondinskiy biosphere reserve, vicinity of 'Agutsa' Forest Station, 49°39′55.03″N 111°25′39.42″E, 1115 m alt., S-facing rocky slope with granitic gneissic rocks near the Station, 20 July 2008 L. Yakovchenko (VBGI); "Gornaya Step" Protected Area, division of Sokhondinskiy biosphere reserve, 14 km S of Kira Village, 49°24′N 112°03′E, 970 m. alt., vicinity of 'Gazultiy' Forest Station, 'Sukhaya Padj' locality, Multiherb prairie with fragments of kharganat-type of vegetation on the S-facing rocky slope, 03 August 2005 Yakovchenko (VBGI); **Zabaikalsky Territory (kray)**, Ononskiy District, road from Verkhniy Sharanay Village to Undino Posel'e Village, 3.5 km W of Zarya

Village, 51°08′51.10″N 116°01′35.70″E, 606 m alt., steppe S-facing slope with shrubs and rocks, 01 August 2008 Yakovchenko (VBGI); Tsasucheyskiy District, road from Nizniy Tcasuchey to Aginskoe, 500 m N of the Bridge on Onon River, vicinity of "Krasny Bator" nature monument, 50°31′45.00″N 115°01′19.10″E, 631 m alt., steppe slope with granitic rocks, 30 July 2008 Yakovchenko (VBGI); Akshinskiy District, 11 km NE of Mogoytuy Village and 10 km N of the junction to Nizniy Zasuchey, 50°24′35,10″N 113°58′04,40″E, 768 m alt., vegetation of kharganat-type (cereals – *Filifolium* – herb steppe with *Armeniaca sibirica*) and shale rocks on S-facing slope, 28 July 2008 Yakovchenko (VBGI); Krasnokamenskiy District, 6.9 km NNE of Tselinniy Village, 50°19′00.60″N 118°08′41.30″E, 710 m alt., Spurs of Klichkinskiy Range, steppe top of spur with granitic gneissic rocks, 05 August 2008 Yakovchenko (VBGI). **CHINA. Neimenggu Province**, Duolun county, Shisanlitan, 42°02.560′N 116°17.014′E, 1324 m alt., on rock, growing together with *Caloplaca subsoluta*, 2009 Hur CH090318 (KoLRI 10932); CH090321 (KoLRI 10935); CH090322 (KoLRI 10936); 42°01.011′N 116°17.738′E, 1434 m alt., on rock growing together with *Protoparmeliopsis muralis*, 2009 Hur CH090332 (KoLRI 10946); Xilin county, Mountain, 43°36.272′N 116°43.242′E, 1260 m alt., on rock, 2009 Hur CH090347 (KoLRI 10961), CH090352 (KoLRI 10966); growing together with *Candelariella* sp. and *Rhizoplaca chrysoleuca* damaged by *Zwackhiomyces zarei*, CH090357 (KoLRI 10971). **UZBEKISTAN.** Along Dugave River, 23 August 1950 N. Shafeev 97 (MSK-L).

Molecular phylogeny — Our ITS rnDNA analyses (Pl. 1) show *Sedelnikovaea baicalensis* as closely related to *Steinia* Körb. and *Pycnora* Hafellner (*Lecideaceae* or *Lecanoromycetidae* incertae sedis), while our mt DNA analyses (Pl. 2) place the species with *Boreoplaca* Timdal (*Lecanoraceae*)

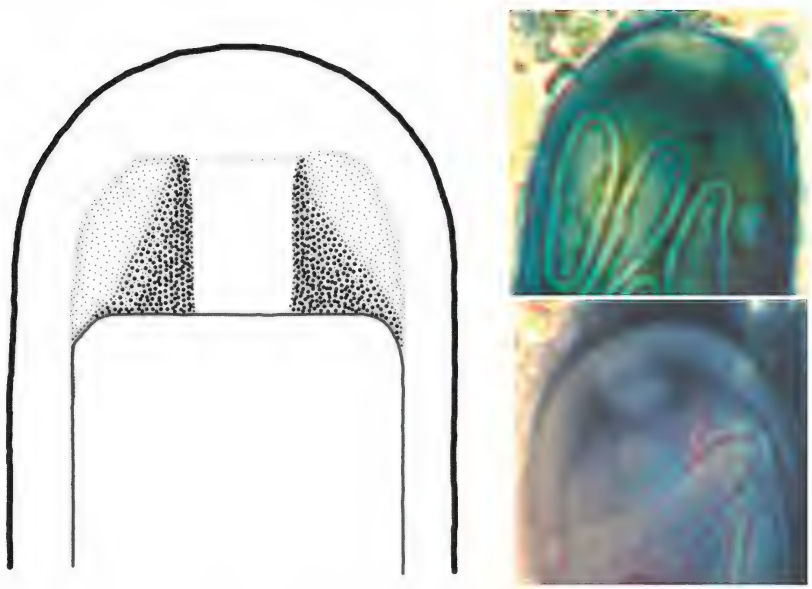

Plate. 3. Asci of *Sedelnikovaea* type.

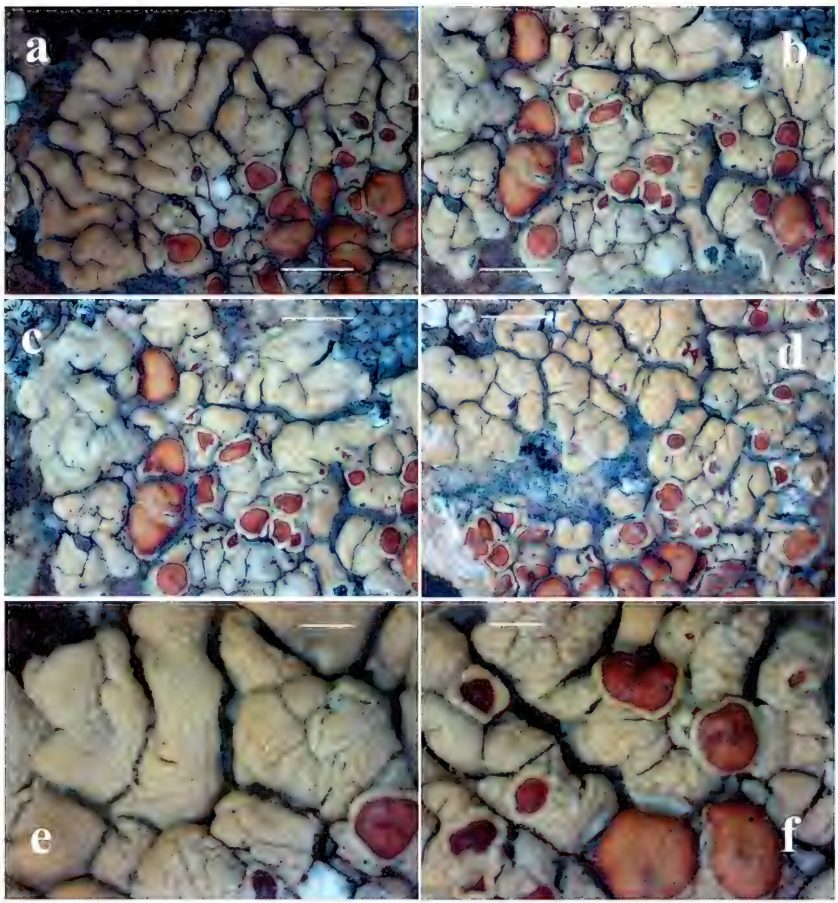

Plate. 4. *Sedelnikovaea baicalensis*. a–d. General habit; e. Enlarged portion of thallus; f. Apothecia. Scale bars: a–d = 3 mm; e, f = 1 mm.

and *Lobothallia* (Clauzade & Cl. Roux) Hafellner (*Megasporaceae*). Our combined ITS + 12S mt SSU phylogenetic analysis suggest that *Sedelnikovaea* is most closely related to genera representing several different families: *Boreoplaca* (*Lecanoraceae*), *Steinia* (*Lecideaceae* or *Aphanopsidaceae*), *Pycnora* (*Lecideaceae*), and *Lobothallia* (*Megasporaceae*). Unfortunately GenBank does not contain both nuclear and mitochondrial sequences for the same species in each of the four genera so that we can only infer that the new genus is unique. Nonetheless, our data confirm that *Sedelnikovaea* is only distantly related to *Protoparmeliopsis*.

Type of ascus — The *Sedelnikovaea*-type ascus shares some characters with the *Lecanora*-, *Candelariella*-, and *Biatora*-type asci. The *Sedelnikovaea*-type shares the strongly thickened apex with a K/I+ pale blue apical dome and a broad K/I– apical cushion found in the *Lecanora* and similar *Candelariella* types (Edwards et al. 2009). Furthermore, the apical cushion is surrounded by a narrow, deeply K/I+ blue zone as seen in the *Biatora*-type (Printzen & Coppins 2009) (Pl. 3). Unlike the *Candelariella*-type, *Sedelnikovaea* asci have only a narrow deeply K/I+ blue zone around the basal part of the apical cushion, unlike the uniformly deep blue apical cushion of *Candelariella*. The *Biatora*-type ascus is distinguished by a narrowly conical K/I– apical cushion that contrasts with the broad cushion found in *Sedelnikovaea*.

As shown above, *Sedelnikovaea baicalensis* shows the highest affinity with *Steinia* and *Pycnora* according to nuclear DNA and with *Boreoplaca* and *Lobothallia* according to mitochondrial DNA. *Pycnora* and *Boreoplaca* are characterized by a *Lecanora*-type ascus (Coppins 2009), while *Megaspora* and *Lobothallia* are characterized by the *Biatora*-ascus type (James & Fletcher 2009). Among the genera mentioned, *Steinia* alone possesses a thin-walled *Aphanopsis*-type ascus (Fletcher et al. 2009). According to Wolseley & Purvis (2009) the *Aphanopsis* ascus is similar to the *Trapelia*-type, but this possibly requires confirmation with fresh material. *Aphanopsis* and *Steinia* represent a separate family, *Aphanopsidaceae*. Finally, we should note that correlation between ascus type and DNA-phylogeny is generally poor in the *Lecanorales* (Ekman et al. 2008).

Our data on Russian, Uzbek, and Chinese material of *Sedelnikovaea baicalensis* completely agrees with the description published in the 'Handbook of the Lichens of the USSR' (Kopachevskaya 1971, as *Placolecanora baicalensis*), with the exception that the asci we measured were much wider: (13–)15–18 (–20) µm vs. 8.5–10.2 µm according to Kopachevskaya (1971).

Sedelnikovaea baicalensis usually grows on open, well-illuminated rock surfaces where it is often associated with *Dimelaena oreina*, *Lecanora frustulosa, Pleopsidium chlorophanum*, *Aspicilia transbaicalica, Aspicilia cinerea, Candelariella vitellina*, and *Rhizoplaca chrysoleuca*.

Additional molecular analyses of *Protoparmeliopsis* and *Sedelnikovaea* will most likely reveal further species that belong in *Sedelnikovaea*.

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A new species of *Leptostroma* on *Pinus henryi* from China

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Abstract — A coelomycetous fungus found on dead fallen secondary needles of *Pinus henryi* from Shennongjia forestry region of Hubei Province, China, is described, illustrated, and discussed. Habitat, morphology, and conidial development place it in the *Rhytismataceae*. It differs from other members of this family on the same substratum by having conidiomata with upper walls more than one cell thick, and is formally named as *Leptostroma magnum* sp. nov. Its conservation status is evaluated as Data Deficient. The type specimen is deposited in the Reference Collection of Forest Fungi of Anhui Agricultural University, China (AAUF).

Key words — anamorph, anatomy

Introduction

During a study of microscopic fungi associated with trees in the Shennongjia forestry region (Hubei, China) in 2010, an interesting coelomycetous species was found on dead fallen needles of *Pinus henryi*. The fungus was collected again in repeat visits to the same site made in 2012 and 2013. It is described, illustrated, and discussed below.

Materials & methods

Specimens were collected from the leaf litter of *Pinus henryi* trees, which were more than 20 years old in 2010. All collections were made at the same location, at an altitude of 1320 m, in Shennongjia forestry region, Hubei, China, in July 2010, October 2012, and April 2013. Specimens were deposited in in the Reference Collection of Forest Fungi of Anhui Agricultural University, Hefei, China (AAUF). For microscopic examination, only needles with sporulating conidiomata were used. External features and arrangement of the conidiomata and associated zone lines were observed with a dissecting microscope at 10–50× magnification. Specimens were rehydrated in water for 10 minutes, and 10–15 μm thick vertical transverse sections of conidiomata were

made using a freezing microtome (YD-202, China) and mounted in 0.1% (w/v) cotton blue with lactic acid. Measurements and drawings of conidiogenous cells and conidia were made from squash mounts in 5% KOH, with more than 30 conidiogenous cells and conidia measured for each specimen. Colors of the various structures were observed in water. Illustrations of external shapes and internal structures of the conidiomata were prepared using the Panasonic XSJ-2 microscope drawing device. Comparisons with other similar species were based on protologue descriptions and illustrations, and, where possible, examination of type material.

Taxonomy

Leptostroma magnum Y.R. Lin & Lan Zhang, **sp. nov.** FIGS 1, 2

MycoBank MB805599

Differs from other species of the *Rhytismataceae* on dead fallen secondary needles of diploxylon pines by having subcuticular conidiomata with upper walls more than one cell thick.

TYPE: China, Hubei, Shennongjia forestry region, Guanmenshan, alt. 1320m, on dead needles of *Pinus henryi* Mast. (*Pinaceae*), 10 July 2010, Y.R. Lin, J.L. Chen & Q. Zheng 2455 (**Holotype,** AAUF 68563).

ETYMOLOGY: *magnum* (Latin = large), referring to the size of the conidiomata.

ZONE LINES black, thin, frequent, with the black fungal tissue penetrating the stele. CONIDIOMATA on both surfaces of the needle, though usually much more frequent on the abaxial side, scattered, sometimes confluent in groups of two or three (especially longitudinally), 350–740 × 110–240 µm, oblong or elliptical, rugged, with a clearly marked outline, shiny black, slightly raising the substratum surface, opening by at least four more or less circular ostioles and one to several irregular tears in the upper wall. IN VERTICAL TRANSVERSE SECTION located underneath the needle cuticle and above the epidermal cells which are largely intact and yellow-brown to brown, with some hypodermal cells invaded by dark grey-brown angular fungal hyphae 2.5–4.5 µm diam. and others strongly brown perhaps as a result of accumulated tannins; somewhat domed, 70–80 µm deep, with an upper wall 8.5–13 µm thick, composed of blackish-brown angular-aliform thick-walled cells below the needle cuticle, becoming slightly pale (yellow-brown) and thin-walled towards the edge and not connecting to the basal wall which is extremely poorly developed. SUBCONIDIOGENOUS LAYER colourless, 6–9.5 µm thick, consisting of textura angularis composed of cells 2–3.5 µm diam. with rather thick walls; a similarly structured but grey-brown semicircular raised area 40–70 µm wide sometimes also present in the centre. CONIDIOPHORES not observed. CONIDIOGENOUS CELLS colourless, thin-walled, smooth, either ampulliform or cylindrical and tapering towards the apex, 12.5–20 × 2.5–4 µm, usually proliferating sympodially, rarely percurrently. CONIDIA 7.5–11.5 × 0.9–1.3 µm, colourless,

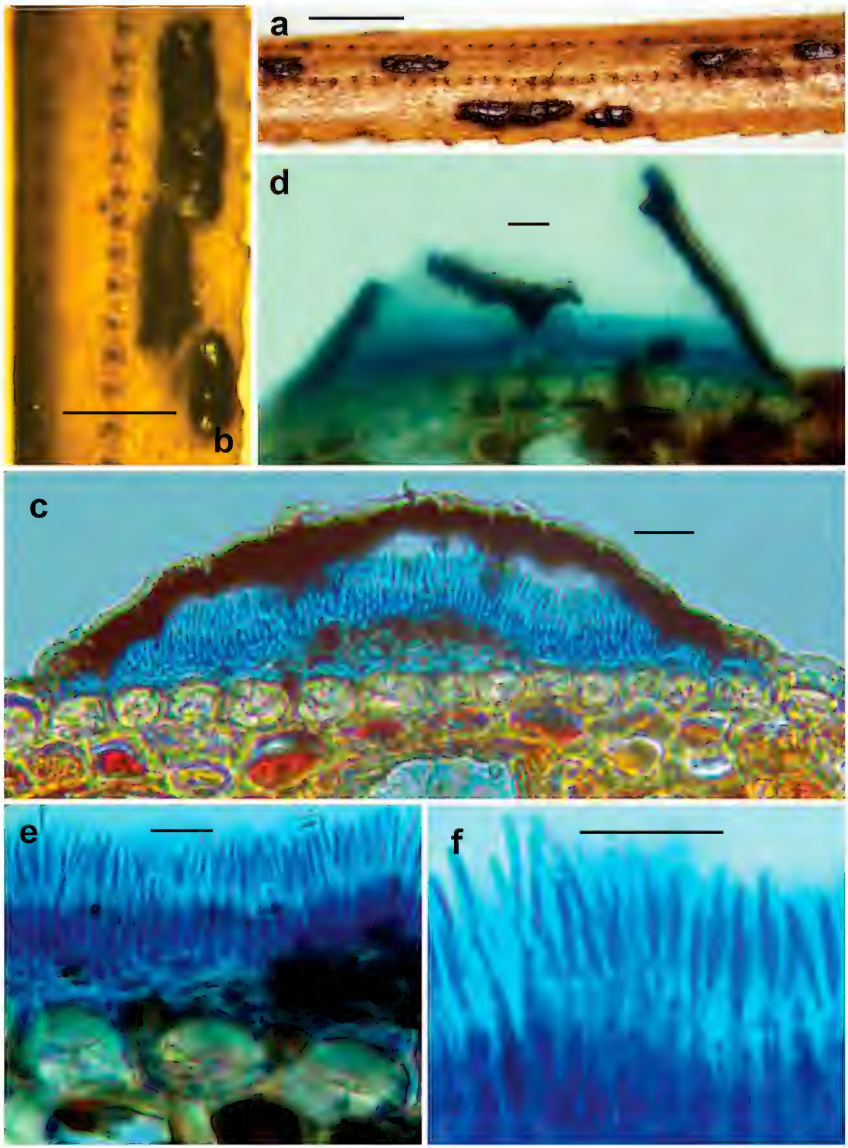

FIGURE 1. *Leptostroma magnum* (ex holotype AAUF 68563): a. Conidiomata on a needle. b. Detail of conidiomata. c. Conidioma in vertical section. d. Opening conidioma in vertical section. e. Conidiogenous cells, conidia, subconidiogenous layer and basal wall. f. Enlargement of conidia. Bars: a, b = 500 µm; c, d = 20 µm; e, f = 10 µm.

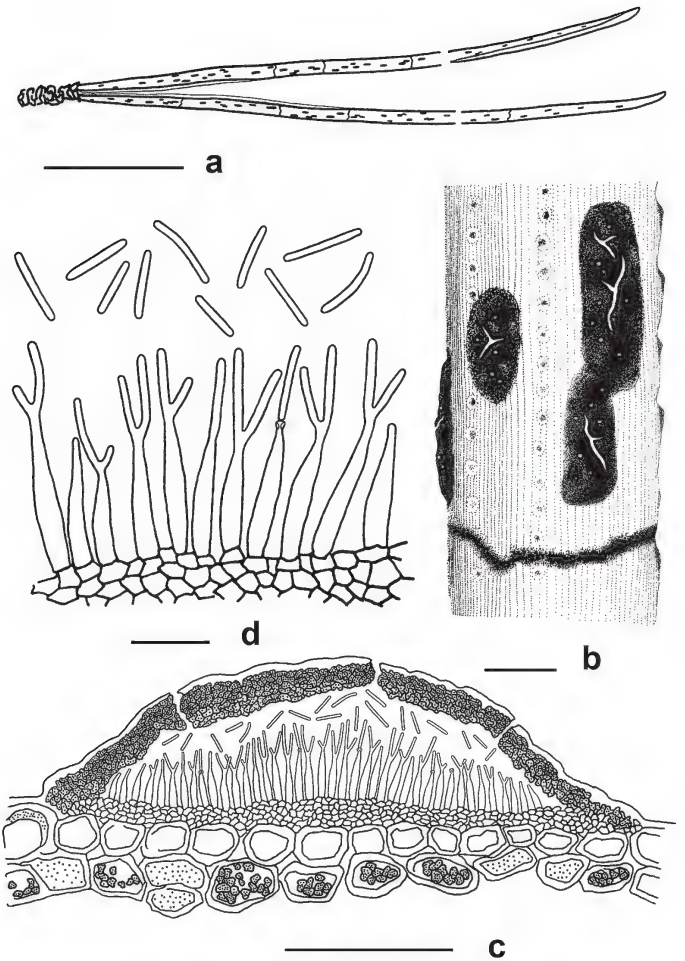

Figure 2. *Leptostroma magnum* (ex holotype AAUF 68563): a. Habit on needles. b. Detail of conidiomata and a zone line. c. Conidiomata in vertical section. d. Conidiogenous cells and conidia. Bars: a = 10 mm;, b = 250 µm; c = 50 µm; d = 10 µm.

aseptate, smooth, cylindrical, sometimes slightly curved, obtuse or round at the ends. Trichogynes not observed. Teleomorph not observed.

Ecology: The fungus was observed on 2–3-year old secondary needles, which had turned yellow-brown to straw yellow, died, and fallen into the litter.

Additional specimens examined: On *Pinus henryi*: **China**, **Hubei**, Shennongjia forestry region, alt. 1320 m, October 2012, L. Zhang & S.J. Wang 2864 (AAUF 68972); April 2013, Y.R. Lin & Q. Li 2971 (AAUF 69079).

Discussion

From their habitat and general form, there can be little doubt that the zone lines and conidiomata in these collections are of a member of the ascomycete family *Rhytismataceae* Chevall. These fungi are seen on dead leaves, twigs, and sometimes other woody material of a wide range of plants. Some are plurivorous. Others are associated with only one or a few closely related species or genera of plants. Many exist as symptomless endobionts in living plant tissues. Some may be mutualistic. Others can be parasitic. A few are pathogens. Fruitbodies are only seen after death of colonized plant tissue. Zone lines defining the extent of individual colonies occur in many species. In some only a teleomorph is observed. In many, perhaps most, an anamorph precedes and accompanies the teleomorph. In a few an anamorph is produced but no teleomorph. Species of this family occurring on pines are generally not found on other plants.

The biology of this fungus poses interesting questions. Why is it only known as an anamorph? Is it possible that the teleomorph has simply not yet been found? In many of the *Rhytismataceae* on pine needles, the anamorph is produced before the teleomorph and, at the correct time of year, the anamorph can be seen but not the teleomorph. The time in this condition is generally rather short. Ascomatal initials usually appear within a month of the first conidiomata appearing (Minter 1977). The three collections of the current species were made at very different times of year, and none had evidence of a teleomorph, so this may be a species with no teleomorph. That would have significant implications for the fungus.

In general, for pine needle inhabiting members of the *Rhytismataceae*, ascomata on dead needles release ascospores, which disperse and colonize young living needles. The fungus then lives there as a parasite or symptomless endobiont until death of the colonized tissues. Then new conidiomata and/or ascomata form, completing the life cycle. Most anamorphs of the *Rhytismataceae* are thought to have a spermatial function (Jones 1935): they are there for sexual reproduction, not dispersal. The conidiomata, conidiogenous cells, and conidia of the present fungus look very similar to many of those anamorphs. If this fungus does not produce ascomata (the usual dispersal state in the *Rhytismataceae*), then what function do these conidiomata have, and how does the fungus colonize new substrata? The search for answers only raises further questions. Does this fungus only occur on *P. henryi*, or is it also on other pines nearby? If so, perhaps there is some reason why the fungus only produces conidiomata on *P. henryi* (for example some interaction within the needle may suppress teleomorph formation), and perhaps the fungus produces the teleomorph on those other pines. Alternatively, the fungus may occur only

on *P. henryi*, and may as a result have evolved different strategies to spread. One possible way might be if the fungus were systemic within the tree, i.e. not just in the needles but also in living twigs. At least one pine needle inhabiting member of the *Rhytismataceae*, *Elytroderma deformans* (Weir) Darker, has adopted that strategy (Lightle 1954). Such a fungus could at least theoretically spread as a symptomless endobiont of seeds. It is clear that the current fungus offers several interesting lines of investigation.

To establish its identity, the current fungus must be compared with other known species. Many members of the *Rhytismataceae* are known from pines, and more than 40 species have been observed on needles. Some fruit on dead portions of still attached living needles (species of the genera *Bifusella* Höhn., *Canavirgella* W. Merr. et al., *Davisomycella* Darker, *Elytroderma* Darker, *Lophodermella* Höhn., *Ploioderma* Darker, *Soleella* Darker). The present fungus, collected on dead fallen needles is very unlikely to be one of them.

Some of the litter-inhabiting species have no known anamorph (*Lophodermium canberrianum* W. Stahl ex Minter & Millar, *L. durilabrum* Darker, *L. erlangshanense* Y.G. Liu, *L. indianum* Suj. Singh & Minter, *L. macci* Sokolski & Bérubé, *L. maximum* B.Z. He & D.Q. Yang, *L. puerense* C.L. Hou & M. Piepenbr., *L. sichuanense* D.X. Qiu & Y.G. Liu, *Meloderma sharmarum* P.F. Cannon & Minter). The present fungus, being known only as an anamorph, is also unlikely to be one of these species.

Although exceptions are known, there is a tendency for species of the *Rhytismataceae* on pine needles to occur on either haploxylon pines (i.e. species with one vascular bundle per leaf) or diploxylon pines (species with two vascular bundles per leaf), but not both (Minter 1977). As the current fungus was found on a diploxylon pine, it is likely to differ from species known on haploxylon pines (*Lophodermium anhuiense* Y.R. Lin, *L. confluens* Y.R. Lin et al., *L. ellipticum* Y.R. Lin, *L. himalayense* P.F. Cannon & Minter, *L. kumaunicum* Minter & M.P. Sharma, *L. mirabile* Y.R. Lin, *L. nitens* Darker, *L. orientale* Minter, *L. pini-bungeanae* Y.R. Lin, *L. pini-excelsae* S. Ahmad, *L. pini-sibiricae* C.L. Hou & S.Q. Liu).

Of the species known to have an anamorph and to occur on secondary needles of diploxylon pines, the following have conidiomata immersed beneath the epidermis: *Leptostroma pineae* (Bubák) Arx, *Lophodermium australe* Dearn., *L. baculiferum* Mayr, *L. conigenum* (Brunaud) Hilitzer, *L. guangxiense* Y.R. Lin, *L. iwatense* Sakuyama, *L. pinastri* (Schrad.) Chevall., *L. ravenelii* Minter, *L. seditiosum* Minter et al., *L. staleyi* Minter, *L. yuexiense* C.L. Hou et al. As the depth of embedding of conidiomata is a rather constant character at species level in the *Rhytismataceae* (Minter 1980), all of those species are also likely to be different from the current fungus, which has subcuticular conidiomata.

There remain four members of the *Rhytismataceae* known to produce subcuticular conidiomata on fallen secondary needles of diploxylon pines: *Lophodermium molitoris* Minter, *L. pini-mugonis* C.L. Hou & M. Piepenbr., *L. yanglingense* Z.M. Cao & C.M. Tian, *Meloderma desmazieresii* (Duby) Darker. All of them have conidiomata with either no upper wall, or with an upper wall only one cell thick. This is another rather constant species-level character in conidial states of the *Rhytismataceae*, and distinguishes those four species from the present fungus that produces conidiomata with upper walls several cells thick.

A few other names in the *Rhytismataceae* have been used for species inhabiting pine needles: *Cryocaligula hedgcockii* (Dearn.) Minter, *Leptostroma ahmadii* Petr., *L. austriacum* Oudem., *L. durissimum* Cooke, *L. decipiens* Petr., *L. pinorum* Sacc., *L. rostrupii* Minter, *L. strobicola* Hilitzer. These are all in genera traditionally considered as anamorphic, and are all known synonyms of species already discussed above. The present fungus thus seems different from all previously described members of the *Rhytismataceae* known from pines, because of its unique combination of characters: it inhabits dead fallen secondary needles of a diploxylon pine, producing subcuticular conidiomata where the upper wall is more than one cell thick.

Fallen needles of pine are known to be a hotspot substratum for sympatric evolution of the *Rhytismataceae* (Burnett 1983): they provide a remarkable range of habitats (the location of a needle on the tree can be significant, needles may be retained by a tree for different numbers of years, and they may die for different reasons). Subtle morphological variations can provide clues to the presence of different species, and molecular evidence is starting to show cryptic speciation where there is little or no morphological variation. There is also an increasing body of evidence that some members of the *Rhytismataceae* are highly specific in their plant associations. It is not surprising therefore to encounter new species in this environment.

Unfortunately, it is generally impossible to predict the teleomorphic genus to which conidiomata belong purely from appearance. Molecular techniques may help to resolve this dilemma. At least one species on pine needles recently found and known only as an anamorph (with subepidermal conidiomata) is being described in *Lophodermium* on the basis of molecular evidence (Koukol & Pusz, pers. comm.). Without molecular information, the only realistic option still remains to place the species in an anamorphic genus.

There are several anamorphic genera in the *Rhytismataceae*. Those accepted in the tenth edition of Ainsworth & Bisby's Dictionary of the Fungi (Kirk et al. 2008) are *Conostroma* Moesz, *Crandallia* Ellis & Sacc., *Cryocaligula* Minter, *Hysterodiscula* Petr., *Leptostroma* Fr., *Melasmia* Lév., and *Tryblidiopycnis* Höhn.

Conidiogenous cells in all these genera are more or less compatible with the present species in shape, colour, size, and developmental characteristics. Ways in which the genera differ are reviewed in the following paragraph.

The type of *Conostroma* is the anamorph of *Colpoma quercinum* (Pers.) Wallr. It characteristically produces rather large multiloculate stromatic conidiomata in bark on twigs of *Quercus* (Sutton 1980). The type of *Crandallia* is the anamorph of *Bifusella acuminata* (Ellis & Everh.) Bonar & W.B. Cooke. It produces multiloculate conidiomata with an upper wall only one cell thick on dead leaves and dead portions of living stems of *Juncus* (Sutton 1980). The type of *Cryocaligula* is the anamorph of *Ploioderma hedgcockii* (Dearn.) Darker. It produces conspicuous unilocular conidiomata with upper walls several cells thick on dead portions of attached living needles of *Pinus*. It is also unusual in having large 1-septate characteristically snowshoe-shaped conidia with possibly a dispersal function, rather than the small aseptate conidia with a spermatial function found in most other anamorphs of the *Rhytismataceae* (Minter 1985). *Hysterodiscula* was originally described with two species, *H. empetri* Petr. and *H. kalmiae* Petr. Index of Fungi (1947) selected *H. empetri* as the generic type, and this choice was endorsed by Sutton (1977, 1980). Based on the information in Sutton (1980), conidiomata in this genus have an upper wall several cells thick, and large 0–1-septate cylindrical to clavate conidia each with a truncate base. The lectotype of *Leptostroma* is *L. scirpinum* Fr., the anamorph of *Hypohelion scirpinum* (DC.) P.R. Johnst. It produces uniloculate often coalescing conidiomata with an upper wall only one cell thick on dead stems usually of *Schoenoplectus lacustris* (L.) Palla (Sutton 1980). The type of *Melasmia* is the anamorph of *Rhytisma acerinum* (Pers.) Fr. It produces multiple conidiomata inside large black stromata on dead portions of living leaves and on dead attached or recently fallen leaves of *Acer*. The conidioma upper wall is only one cell thick. The type of *Tryblidiopycnis* is the anamorph of *Tryblidiopsis pinastri* (Pers.) P. Karst. It produces large complex often multilocular conidiomata with upper walls only one cell thick on twigs of *Picea*. Its conidia are long, thin and curved.

From this review, it is evident that the present species has a combination of characters not perfectly matched by any one of these genera: those producing conidiomata with thick upper walls are on woody material or have large septate conidia with a dispersal function; those on leaves produce conidiomata with thin upper walls and have conidia with a spermatial function. In external appearance, and in shape, size and development of conidiogenous cells and conidia, the present fungus looks like a species of *Leptostroma*. This is by far the largest anamorphic genus of the *Rhytismataceae*, and it has already been used for anamorphs of many members of the family inhabiting pine needles (Minter

1980; Sutton 1980; Cannon & Minter 1986; Lin et al. 2012). The only significant difference between the present fungus and *L. scirpinum*, the type of the genus, is that its conidiomatal upper walls are several cells thick.

Closer inspection of other pine needle inhabiting *Leptostroma* anamorphs shows that there is, in fact, considerable variation in conidiomatal upper walls. Where conidiomata are subepidermal, the upper wall is often paler, may be limited to an area round each ostiole (where present), or may even apparently be absent. Where conidiomata are subcuticular, the upper wall is often much blacker and much more extensive. The extent and pigmentation of conidiomatal upper walls may therefore be related, at least partly, to depth of embedding in the plant tissue, and that may reflect water availability and the need to protect against UV radiation (Sherwood 1981). In the present species, where the conidiomata are subcuticular, the upper wall is even more strongly developed. Until the biological implications of a thicker upper wall are better understood, upper wall thickness seems insufficient reason to establish a new genus, the fungus is therefore formally named below as a new species of *Leptostroma*.

Conservation status

Data Deficient. The species is poorly known and has been recorded from only one location (in a protected area which is part of the UNESCO Man & Biosphere network). If an obligate association with *P. henryi* were to be demonstrated, the conservation status of this fungus would automatically be at least Near Threatened, the current IUCN red list conservation status of its only known associated plant.

Acknowledgments

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A new *Stemonitis* species from Turkey

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Abstract — *Stemonitis mediterraneensis* is described herein as a new species of myxomycete from material collected on a rotten wood remnant of *Abies cilicica* subsp. *cilicica* from Adana-Aladağ in Turkey. The new species is characterized by rough warty pale brownish to yellowish-brown spores and smooth capillitia lacking spines.

Key words — *Mycetozoa*, *Protista*, slime moulds, *Stemonitidaceae*

Introduction

Myxomycetes are a small, relatively homogeneous group of eukaryotic organisms (Stephenson & Stempen 1994). They occur on a variety of substrates such as living plants as well as decaying logs, dead leaves, and other organic matter (Alexopoulos et al. 1996). Since Gleditsch (1753) first described *Stemonitis* Gled., 17 species have been identified (Lado 2005-2014), including nine recorded in Turkey (Sesli & Denchev 2014). Recent morphological examination has revealed a new species, *S. mediterraneensis,* bringing world's total *Stemonitis* species to 18.

Materials & methods

Morphological observations and measurements were conducted under a Leica Z 6 APO-A stereoscopic microscope (Leica Microsystems CMS GmbH, Mannheim, DE). The total height of the sporocarps as well as height and width of the sporotheca were measured. Whenever possible, 15 sporocarps of each collection were checked (110 sporocarps in total). Microscopic characteristics were observed and measured from material mounted in Hoyer's medium under a Leica DM-750 light microscope. A total of 110 spores (including ornamentation) were measured using an oil immersion objective; spore color and ornamentation also were determined. SEM images were prepared with a Zeiss DSM-950 scanning electron microscope (Micronova, Espoo, Finland). Specimens are conserved in the Fungarium, Mushroom Application and Research Center, University of Selçuk, Konya, Turkey (KONF).

Taxonomy

Stemonitis mediterraneensis H.H. Doğan & Eroğlu, **sp. nov.** FIGURE 1

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Differs from *Stemonitis mussooriensis* by its smaller spores with shorter rougher warts.

TYPE: Turkey. Adana: Aladağ, Meydan Yaylası, Ceritler, 37°29′01″N, 035°19′45″E, 1265 m, on rotten wood of *Abies cilicica* (Antoine & Kotschy) Carrrière subsp. *cilicica*, 27 Oct. 2011, leg. H.H. Doğan 9315 (**Holotype**, KONF HD 9315; **Isotype**, KONF GE 560).

ETYMOLOGY: referring to the Mediterranean region where the specimens were collected.

SPOROCARPS in groups, cylindrical, total height 2.2–3.5 mm, dark brown. Sporotheca 1.5–2.8 mm in length and 0.2–0.4 mm in diameter. STALK short, about 1/2–1/3 of the total height, 0.7–0.8 mm in length, shining, dark brown. PERIDIUM fugacious. COLUMELLA 7–8 µm wide, cylindrical, dark brown, opaque, similar in color to the stalk, tapering gradually upwards and merging into the capillitium just below the apex. CAPILLITIUM 1–1.5 µm in diameter, pale brown, with a surface net consisting of variable-sized meshes (4–110 µm), smooth, not bearing spines and the internal reticulum 3–4 meshes over the radius; the surface net delicate with angular meshes and attached to the columella. SPORES (8.5–)9–10.6 µm in diameter, globose, covered with rough warts, pale brownish, yellowish brown under the light microscope. By SEM, the ornamentation consisting of regularly distributed and isolated spiny warted 0.3 µm long projections. HYPOTHALLUS discoid under the separate sporangia, yellowish brown.

ECOLOGY & DISTRIBUTION: Found on rotten wood of *Abies cilicica* subsp. *cilicica* in a mixed forests of *A. cilicica* subsp. *cilicica* and *Cedrus libani* A. Rich.

Discussion

Stemonitis is characterized by stalked cylindrical sporocarps, an evanescent peridium, a columella that almost reaches the top of the sporocarp, a pale brown, red brown or dark brown capillitium that arises from the entire length of the columella, the absence of capillitial spines, spores that are dark brown, rust brown or lilac grey in mass and usually pale brown in transmitted light.

Morphologically and anatomically, six species are very close to *Stemonitis mediterraneensis;* the seven similar species can be distinguished as follows:

Key to *Stemonitis mediterraneensis* and related species

1. Spores reticulate 2
1. Spores with isolated warts or blunt spines 3
2. Spores usually ≤8 µm ..... *S. virginiensis*
2. Spores usually ≥8 µm ..... *S. fusca*
3. Spores usually ≤6 µm ..... *S. axifera*
3. Spores usually ≥6 µm 4

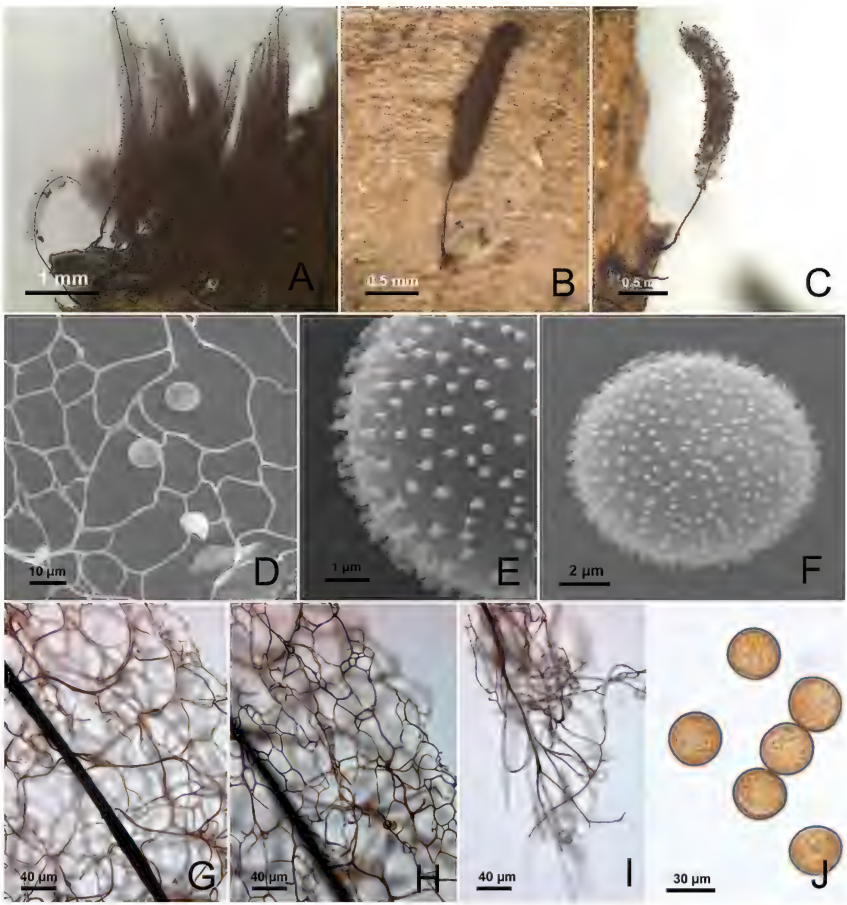

FIGURE 1 – *Stemonitis mediterraneensis* (holotype). A: Sporocarps (SM). B, C: An entire sporocarp (SM). D: Capillitial meshes and spores (SEM). E: Detail of spore ornamentation (SEM). F: Spores (SEM). G, H: Capillitial meshes (LM). I: Expanded columella apices (LM). J: Spores (LM).

4. Surface net small-meshed 5
4. Surface net large-meshed 6
5. Stalk 1/4–1/3 of the total height .....*S. pallida*
5. Stalk 1/5–1/4 of the total height, sometimes almost lacking.....*S. herbatica*
6. Spores bearing fine pale warts.....*S. mussooriensis*
6. Spores bearing rough spiny warts..... *S. mediterraneensis*

Its rough warty spores distinguish *Stemonitis mediterraneensis* from *S. fusca* Roth and *S. virginiensis* Rex, both characterized by reticulate spores with rows

of spines, some of which are connected by ridges (Nannenga-Bremekamp 1991, Ing 1999). *Stemonitis mediterraneensis* is close to *S. axifera* (Bull.) T. Macbr., *S. pallida* Wingate, *S. herbatica* Peck, and *S. mussooriensis* G.W. Martin et al. in its spore ornamentation, but its spore morphology easily distinguishes the new species. The colorless spores of *S. axifera* are smaller (5-6 µm) and almost smooth to covered with very small, pale warts. The pale rust spores in *S. pallida* are also smaller (6.5–7.5(–8) µm) and ornamented with pale warts, sometimes appearing almost smooth (Nannenga-Bremekamp 1991; Stephenson 2003). In spore size, *S. herbatica* and *S. mussooriensis* are the most closely related species to *S. mediterraneensis* but can be distinguished by the lilac brown color of the spores.

The absence of a spinose surface net, shared by *Stemonitis mediterraneensis* and *S. herbatica,* distinguishes those two species from *S. fusca*, *S. virginiensis*, *S. pallida*, and *S. mussooriensis,* all of which have a spinose surface net. Its unique combination of macroscopic, capillitial, and spore features easily support *S. mediterraneensis* as an independent *Stemonitis* species.

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A new species of *Coccomyces* with dimorphic paraphyses

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Abstract — A new *Coccomyces* species found on fallen leaves of *Millettia dielsiana* at Mt Sanqingshan, Jiangxi province, China, is described as *Coccomyces alienus*. This taxon is distinguished from all other *Coccomyces* taxa by having two types of paraphyses. The type specimen is deposited in the Reference Collection of Forest Fungi of Anhui Agricultural University, China (AAUF).

Key words — foliicolous fungus, taxonomy, *Fabaceae*, fungal diversity

Introduction

Coccomyces De Not. is the second-largest genus in the *Rhytismataceae* (*Rhytismatales, Leotiomycetes, Ascomycota*), of which at least 116 species have been recorded (Kirk et al. 2008, Lin et al. 2012). They are widely distributed and occur on leaves, herbaceous stems, bark, and wood (Sherwood 1980). The study of *Coccomyces* in China began in 1934 with the records of *C. dentatus* on *Quercus* and *Castanea,* and *C. delta* (Kunze) Sacc. on *Lauraceae* (Teng 1934). Subsequently, twenty-eight additional species have been reported (Korf & Zhuang 1985; Lin et al. 1994; Lin 1998; Hou et al. 2006, 2007; Jia et al. 2012; Zheng et al. 2012; Wang et al. 2013; Yang et al. 2013). Of the 159 fungal species known to inhabit *Millettia*, no *Coccomyces* species has yet been reported on this plant genus (Farr & Rossman 2014).

The present study, which is based on a specimen collected from Mount Sanqingshan National Park (Jiangxi Province, China) describes a new species of *Coccomyces* on a *Millettia dielsiana* vine.

Materials & methods

The external shape, size, colour, opening mechanisms of ascomata and conidiomata, and zone lines were observed on dried reference materials using a stereoscope with

10–50× magnification. After rehydration in water for ca 10 min, the fruitbodies were cut into 10–20 µm thick sections with a cryo-microtome. The sections were examined microscopically in water, 5% KOH, Melzer's reagent, or lactophenol-cotton blue. Sections pretreated in water were mounted in lactic acid or cotton blue to observe ascomatal and conidiomatal outlines in vertical section. Colours of various structures and ascospore contents were determined in water. Asci, ascospores and paraphyses were measured and drawn from tissues mounted in 5% KOH. At least thirty asci, ascospores, and paraphyses were measured for each specimen. Figures of the external features and internal structures were drawn using a microscopic painting device. The type specimen is deposited in the Reference Collection of Forest Fungi of Anhui Agricultural University, Hefei, China (AAUF).

Taxonomy

Coccomyces alienus Y.R. Lin & Xiao Y. Wang, **sp. nov.** FIGS 1–6

MycoBank MB 807573

Differs from *Coccomyces dentatus* by triangular to pentagonal ascomata with lip cells and periphysoids, loose-textured and fawn-coloured internal stromatic matrix (textura angularis-globulosa), two types of paraphyses with very different shapes, cylindrical and thinner asci, and narrower ascospores.

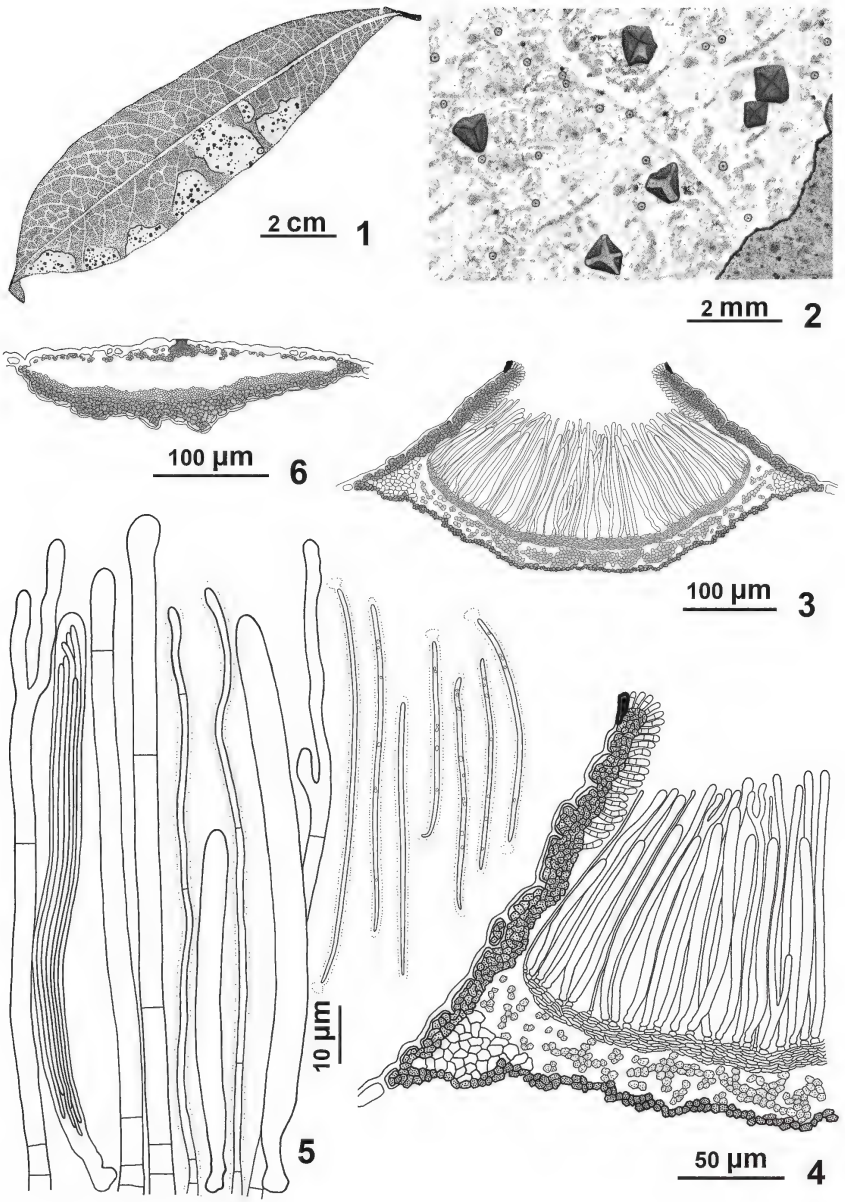
TYPE: China, Jiangxi, Mount Sanqingshan National Park, Bingyudong, alt. 1550 m, on fallen leaves of *Millettia dielsiana* Harms (*Fabaceae*), 21 August 2012, Y.R. Lin, X.Y. Wang & L. Zhang 2661 (**Holotype**, AAUF 68769).

ETYMOLOGY: *alienus* (Latin = alien), referring to two types of paraphyses with very different shapes.

ZONE LINES generally frequent, dark brown or black, thin, entirely or partly surrounding bleached spots of 8–20 mm dam.

CONIDIOMATA in similar positions to ascomata on the host, scattered to clustered. In surface view, conidiomata 110–260 µm diam., rounded or subrounded, black-brown in the centre and the perimeter line of the conidioma, hazel to brown elsewhere, flattened, discharging spores through an apical ostiole. In median vertical section, conidiomata intraepidermal, more or less lenticular. UPPER WALL extremely poorly developed, yellow-brown but black-brown near the ostiole, composed of tiny angular cells beneath the cuticle. Sometimes individual epidermal cells conjoining the cuticle. BASAL WALL 12–25 µm wide, of dark brown textura angularis with thick-walled cells 3–7 µm diam. SUBCONIDIOGENOUS LAYER 10–15 µm wide, comprised of colourless, thin-walled angular cells 2–3 µm diam. CONIDIOGENOUS CELLS and CONIDIA not present in material available.

ASCOMATA developing on both sides of leaves, mostly hypophyllous, scattered or somewhat clustered, sometimes two or three coalescent; occurring in subcircular or irregular pale areas, sandy-brown in colour with obvious edges. In surface view, ascomata 520–900 µm diam., triangular to pentagonal,



Figs 1–6. *Coccomyces alienus* (holotype) on *Millettia dielsiana*. 1. Habit on a leaf. 2. Detail of fruitbodies and a zone line. 3. Ascoma in median vertical section. 4. Portion of ascoma in median vertical section. 5. Paraphyses, asci and ascospores. 6. Conidioma in vertical section.

black-brown to black, slightly shiny, moderately raising above the surface of leaf but flat or slightly hollow in the centre portion, edge defined, with an obvious preformed dehiscence mechanism, opening by 3–5 radial splits which extend nearly to the edge of ascoma to expose the cream yellow top of hymenium. Lips poorly developed. In median vertical section, ascomata intraepidermal. Covering stroma 15–25 µm thick, composed of blackish brown textura angularis-epidermoidea with thick-walled cells 3–5.5 µm diam., connecting to the basal stroma. There is a small thin mulch that comprises strongly carbonized tissue with no obvious cellular structure on top of the covering stroma, sometimes a few epidermal cells connecting with the cuticle. Lip cells hyaline, 5–8 × 2–2.5 µm, cylindrical, slightly swelling at the apex, thin-walled, 0–1-septate. Periphysoids lining the inner face of the covering stroma, 8–15 × 2–3 µm, cylindrical, often indistinctly swollen at the top, hyaline, straight or curved, 1–3-septate. Basal stroma consisting of 1–2(–3) layers of black-brown, angular, thick-walled cells 4–6 µm diam. 25–40 µm thick textura angularis with colorless, thin-walled cells 5–8(–10) µm diam. existing between the covering stroma and basal stroma. Internal matrix of stroma moderately developed, 20–35 µm thick, comprised of loose, gelatinized, nearly colourless or fawn-coloured textura angularis-globulosa with thin-walled cells 4–8 µm diam. Subhymenium 10–15 µm thick, slightly concave, consisting of colourless textura porrecta. Paraphyses exceeding height of asci by 10–20 µm, of two types: A-paraphyses frequent, 3–4.5 µm wide, thick and strong, cylindrical, straight or occasionally curved, mainly gradually or abruptly swollen to 4.5–6 µm at the apex, sometimes branching near the top or at lower positions, thinly septate, smooth-walled, with no gelatinous matrix; B-paraphyses sparse, 0.8–1.4 µm wide, narrow, filiform, slightly curved, occasionally gradually swollen to 1.5–2.5 µm at the apex, unbranched, septate, covered with a ca. 0.5 µm thick mucous coating. Asci ripening sequentially, somewhat sparse, 85–120 × 4.5–6 µm, cylindrical, apex rounded, thin-walled, without a circumapical thickening, short-stalked, J–, 8-spored. Ascospores arranged in a fascicle, 45–75 × 1–1.2 µm, filiform, slightly tapered towards the base, straight or slightly curved, hyaline, aseptate, containing oil drops, surrounded by a 0.5–1 µm thick gelatinous sheath, sometimes with subglobose gelatinous caps at the apex or both ends.

Host species, habitat, & distribution: Producing conidiomata and ascomata on fallen leaves of *Millettia dielsiana*. Known only from the type locality, Jiangxi Province, China.

Comments—*Coccomyces alienus* is very similar to *C. dentatus* (J.C. Schmidt) Sacc. in the way ascomata and conidiomata are embedded, the opening form of fruitbodies, and the lack of excipulum. However, *C. dentatus* has quadrangular to hexagonal ascomata with nonexistent lip cells, periphysoids, and a black

mulch that occurs on top of the stromal covering, a virtually absent internal stromal matrix, monomorphic, shorter (80–105 µm long) paraphyses, shorter and thicker (70–105 × 8–10 µm) cylindric-clavate asci, and thicker (2 µm wide) ascospores (Sherwood 1980).

Coccomyces limitatus (Berk. & M.A. Curtis) Sacc., which produces macroscopically similar zone lines, ascomata, and conidiomata and similarly shaped periphysoids, asci, and ascospores, differs from *C. alienus* in its absence of lips, a well developed excipulum, a covering stroma not extending as far as the basal stroma, a subhymenium of globose cells, and a much thicker (up to 50 µm thick) internal stromal matrix composed of loose hyphae and crystals (Johnston 1986). Sherwood's earlier (1980) observation that the excipulum and periphysoids of *C. limitatus* are absent was contradicted by Johnston (1986), who suggested that the excipulum of *C. limitatus* form after the ascomata open and may not be obvious when immature. Young ascomata have the appearance of a well-developed layer of short, hyaline, cylindric periphyses that lengthen and develop septa to form the excipulum.

Coccomyces cyclobalanopsidis Y.R. Lin & Z.Z. Li, which resembles *C. alienus* in the zone lines, the way the ascomata open, and the shapes and sizes of the asci and ascospores, is distinguished from the new species by its triangular to hexagonal ascomata, covering stroma and basal stroma consisting of textura globulosa, the longer and thicker (12–20 × 3–4 µm) periphysoids, absent lip cells, excipulum arising from the inner layer of the covering stroma, and the extremely poorly developed internal stromal matrix of textura intricata (Lin et al. 2000).

We observed that *C. alienus,* which produced mature or overripe conidiomata and ascomata on recently dead leaves, was often confused with *Terriera minor* (Tehon) P.R. Johnst., whose immature or barely mature ascomata could be easily distinguished on a same leaf, suggesting that *C. alienus* fruits earlier than *T. minor*. This new fungus may be a weak pathogen causing leaf spot. It is possible that it first develops latently within the living leaves but exhibits no obvious symptoms, after which the asexual and sexual stages appear sequentially on the brown lesions of the infected leaves as the bioenergy of the host declines. Thereafter diseased leaves become reddish brown or sandy-brown and fall during July and August. Sherwood's (1980) observation that most *Coccomyces* species fruit on recently dead plant materials is largely consistent with our observations.

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Cytospora from *Salix* in northern China

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Abstract — Fungal specimens from *Salix* causing willow *Cytospora* canker in northern China were evaluated through morphological observation and phylogenetic analysis. They were identified as four species — *C. rostrata* sp. nov., *C. nivea*, *C. chrysosperma*, and *Valsa populina*. *Cytospora rostrata* is a new beaked species parasitizing *Salix cupularis* in Gansu Province, China. It is morphologically distinguished from other *Cytospora* species by its fruiting bodies with a single locule and a thorn-like beak. Molecular evidence obtained from the internal transcribed spacer (ITS) region also suggests that *C. rostrata* represents a distinct lineage within *Cytospora*. This is the first Chinese record of *C. nivea* on *Salix*.

Key words — *Diaporthales*, morphology, phylogeny, taxonomy

Introduction

Willow (*Salix*) is a crucial commercial and ecologically important forest tree that is grown as a major biomass energy source for its yield potential and coppicing ability in Netherland (Londo 2002). In China, willows are one of the most important trees for their economic and ecological value, and are also widely planted in courtyards, residential districts, and parks as a highly adversity-resistant perennial tree. However, most willow trees are under serious threat in northern China from dieback and stem canker caused by *Cytospora* species. A systematic study of pathogenic fungi on *Salix* is badly needed.

The genus *Cytospora* was established by Ehrenberg in 1818 and has been shown to be the anamorph of the ascomycetous genus *Valsa* Fr. (*Diaporthales*) (Adams et al. 2005), causing serious dieback and stem canker diseases commonly found on woody plants (Schreiner 1931, Spielman 1984, Wang et al. 2014). About 110 species of *Cytospora* have been described on more than 85 host species (Adams et al. 2005, Kirk et al. 2008). Traditional taxonomy

of *Cytospora* has been based heavily on host species, but Adams et al. (2006) and Mehrabi et al. (2011) have recently stressed the taxonomic significance of morphology (including presence or absence of a conceptacle, number and arrangement of locules, size and shape of conidiophores, and conidial size) in this group.

Twenty-six species of *Cytospora* and *Valsa* have been recorded as pathogenic fungi of willow canker worldwide, of which nine have been reported in China: *C. capreae* Fuckel, *C. salicis* (Corda) Rabenh., *C. fugax* (Bull.) Fr., *C. fertilis* Sacc., *C. translucens* Sacc., *C. germanica* Sacc., *C. chrysosperma*, *C. ambiens* (Pers.) Sacc., and *V. populina* (Deng 1963, Tai 1979, Wei 1979, Zhuang 2005). However, morphological data for some of these records were inadequate for identification, and taxonomic studies of *Cytospora* species from *Salix* in China are lacking. We therefore collected 116 isolates from ca. 200 specimens on willow trees from nine provinces of northern China and used 14 representative isolates in a systematic study based on morphology and DNA sequence variation. Our specimens included a new taxon, which we describe as *Cytospora rostrata*.

Materials & methods

Sample collection and isolation

A total of 116 *Cytospora* isolates were obtained from lesions on infected twigs of willow trees in nine Chinese provinces: Xinjiang, Tibet, Shaanxi, Gansu, Ningxia, Qinghai, Heilongjiang, Beijing, and Liaoning. Plant tissues were cut into ca. 5 mm diameter discs, placed in 70% ethanol for 1 min, and then rinsed with sterile water three times. The twig segments were then placed onto the surface of potato dextrose agar (PDA) and incubated at 28°C. The growing edges of hyphae were transferred onto a new PDA plate after 1–2 days. Each isolate was purified through single spore isolation and maintained according to Fan et al. (2014).

Morphological observation

Cytospora specimens used for morphological observation have been conserved in the Museum of Beijing Forestry University (BJFC). Morphological characteristics of the fruiting body, including the size of stromata, color, size and shape of disc, presence or absence of a conceptacle, number and diameter of ostioles per disc, arrangement of locules, and conidiophore size and shape, were recorded. Over 20 conidiomata were sectioned and diameters of 50 conidia were randomly chosen to measure the length and width by a Leica light microscope (DM 750). Cultural characteristics including colony color and pycnidial structure were recorded after 3, 7, and 30 days.

DNA extraction and PCR amplification

Genomic DNA of all cultures was extracted using a modified CTAB method (Doyle & Doyle 1990). Extracted DNA was electrophoresed on 1% agarose gel to check genomic DNA quality. The ITS1-5.8S-ITS2 (ITS) region of the rDNA was amplified by PCR using the primer pair ITS1 and ITS4 (White et al. 1990) in 20 µL volumes with 1 µL (20 ng) DNA template, 1.6 µL dNTP (2.5 mmol/L), 2 µL 10×PCR buffer (15 mM/mL $MgCl_2$),

Table 1. New ITS sequences of *Cytospora* and *Valsa* spp. included in phylogenetic analyses.

Species	Host	Location	Isolate	Sequence
C. rostrata	*Salix cupularis*	China, Gansu	BJFC CGLs251 **HT**	KC313890
	S. cupularis	China, Gansu	BJFC CGLs252	KC313891
	S. cupularis	China, Gansu	BJFC CGLs280	KC313892
C. chrysosperma	*S. babylonica*	China, Ningxia	BJFC CNLs2	KC787357
	S. babylonica	China, Shaanxi	BJFC CSLs3	KC787358
	S. carmanica	China, Gansu	BJFC CGLs4	KC787359
	S. cupularis	China, Gansu	BJFC CGLs8	KC787360
	S. matsudana	China, Gansu	BJFC CGLs2	KC787361
	S. matsudana f. *tortuosa*	China, Gansu	BJFC CGLs7	KC787362
	S. babylonica	China, Ningxia	BJFC VNLs2	KC787365
C. nivea	*S. cupularis*	China, Gansu	BJFC CGLs32	KC787364
	S. cupularis	China, Gansu	BJFC VGLs33	KC787363
V. populina	*S. matsudana* f. *tortuosa*	China, Gansu	BJFC VGLs7	KC787366
	S. cupularis	China, Gansu	BJFC VGLs24	KC787367

HT = holotype.

1 µL of each primer (10 µLmol/L), 0.1 µL Taq DNA polymerase (5 U/µL), and 13.3 µL ddH_2O. The PCR protocol comprised denaturation at 94°C for 10 min, followed by 35 cycles of 94°C for 30 s, 51°C for 30 s, 72°C for 40 s, and a final cycle at 72°C for 10 min. PCR products were electrophoresed on 1% agarose gel and the DNA was sequenced by the Shanghai Invitrogen Biological Technology Corporation.

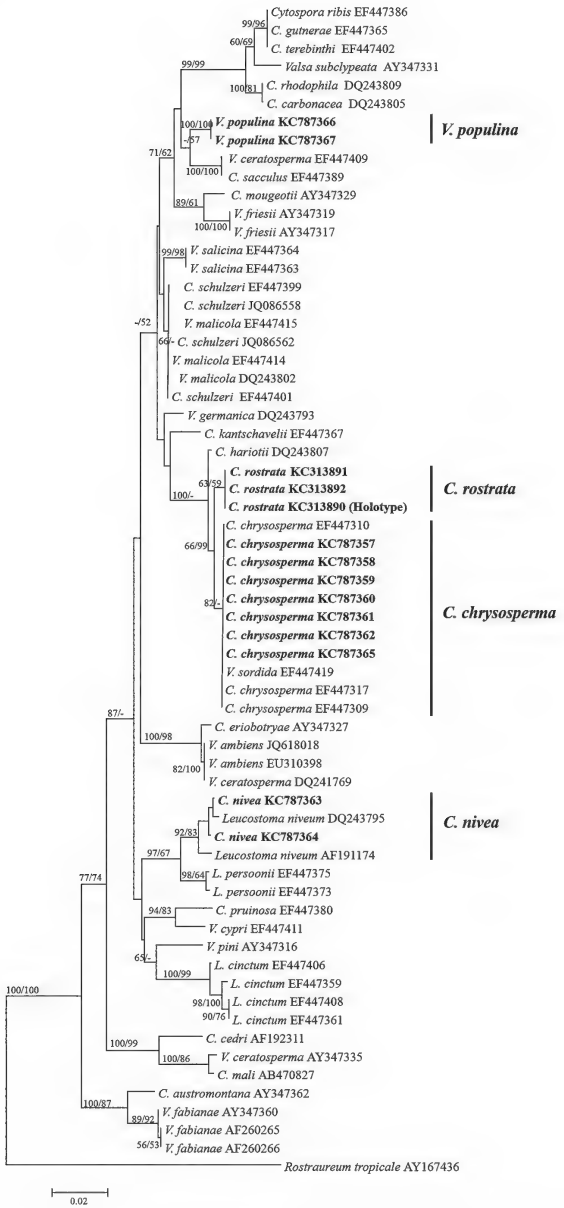
Phylogenetic analyses

Our 14 sequences representing the four species isolated from *Salix* were used in the phylogenetic analyses (Table 1) with 49 GenBank ITS sequences representative of a cross-section of *Cytospora*/*Leucostoma*/*Valsa* spp., and *Rostraureum tropicale* Gryzenh. & Wingf. as outgroup. The sequences were assembled using SeqMan (7.1.0) and aligned using Clustal X (1.81). Alignments were manually adjusted to allow maximum alignment and maximum sequence similarity, and gaps were treated as missing data. The phylogenetic tree was constructed with the neighbor-joining (NJ) method using PAUP (4.10), and the maximum likelihood (ML) method using Phylip (3.6.9) (Swofford 2000, Tamura et al. 2011). The stability of relationships was assessed by bootstrap analyses comprising 1000 data settings. Constructed trees were viewed by Treeview (1.6.6) software.

Results

Phylogenetic analyses

The alignment of ITS regions consisted of 510 base pairs. The ML analysis produced a similar topology to the NJ analysis presented in Plate 1.



Pl. 1 Phylogenetic tree based on aligned sequences of ITS1-5.8S-ITS2 region of 63 isolates of *Cytospora, Leucostoma,* and *Valsa* generated from neighbor joining (NJ) and maximum likelihood (ML) analyses. NJ and ML bootstrap values greater than 50% are indicated (separated by a slash from 1000 heuristic replicates). Tree is rooted with outgroup *Rostraureum tropicale*. The new sequences are represented in bold font.

The sequences grouped into 29 clades. *Cytospora rostrata* had a high similarity of several nucleotides variation with *C. chrysosperma*. However, the phylogenetic tree and the morphological characteristics indicated *C. rostrata* represents an independent taxon.

Taxonomy

Pl. 2 *Cytospora rostrata* (Holotype, BJFC-CGLs251). **a** Conidiomata on twig. **b, c** Transverse sections through conidiomata. **d** Detail of beak on twig. **e, f** Longitudinal sections through conidiomata. **g** Conidiophores and conidiogenous cells. **h** Conidia. **i** Colonies on PDA at 3 d (left) and 30 d (right). Scale bars: a = 1 mm; b–f = 0.5 mm; g = 20 μm; h = 5 μm.

Cytospora rostrata C.M. Tian & X.L. Fan, **sp. nov.** Plate 2

MycoBank MB 810728

Differs from *Cytospora chrysosperma* by its conidial stromata with a single simple locule and thorn-like ostiolar beak.

Type: **CHINA**, **Gansu Province**, Gannan, Diebu, 34°04′48.85″N 103°23′34.20″E, elev. 2989 m asl, on stems of *Salix cupularis* Rehder, 9 August 2012, Y.M. Liang & X.L. Fan (**Holotype**, BJFC-S726; culture BJFC-CGLs251; GenBank KC313890).

Etymology: *rostrata*, referring to the beaked fruiting body.

Conidiomatal stromata immersed in bark, erumpent, beaked, discoid to conical, with a single locule. Disc absent. Beak slim, spiny, 0.87–1.20 mm (x = 0.98, n = 20), with one ostiole per beak. Ostiole in the center of the beak, 22–24 µm (x = 23 µm, n = 20) in diameter. Locules discoid, simple-loculed, 455–851 µm (x = 668 µm, n = 20) in diameter. Conidiophores hyaline, unbranched or occasionally branched at the bases, 15.3–25.1 µm (x = 18.9 µm, n = 20). Conidia hyaline, eguttulate, elongate-allantoid, aseptate, 3.6–4.8 × 1.0–1.6 µm (x = 4.1 × 1.38 µm, n = 50). Cultural characteristics were white originally, became pale yellow after 6–7 d. The colony was flat, thin, and with a uniform texture. The arrangement of conidiomata on PDA was circular in the center and radial to colony edge.

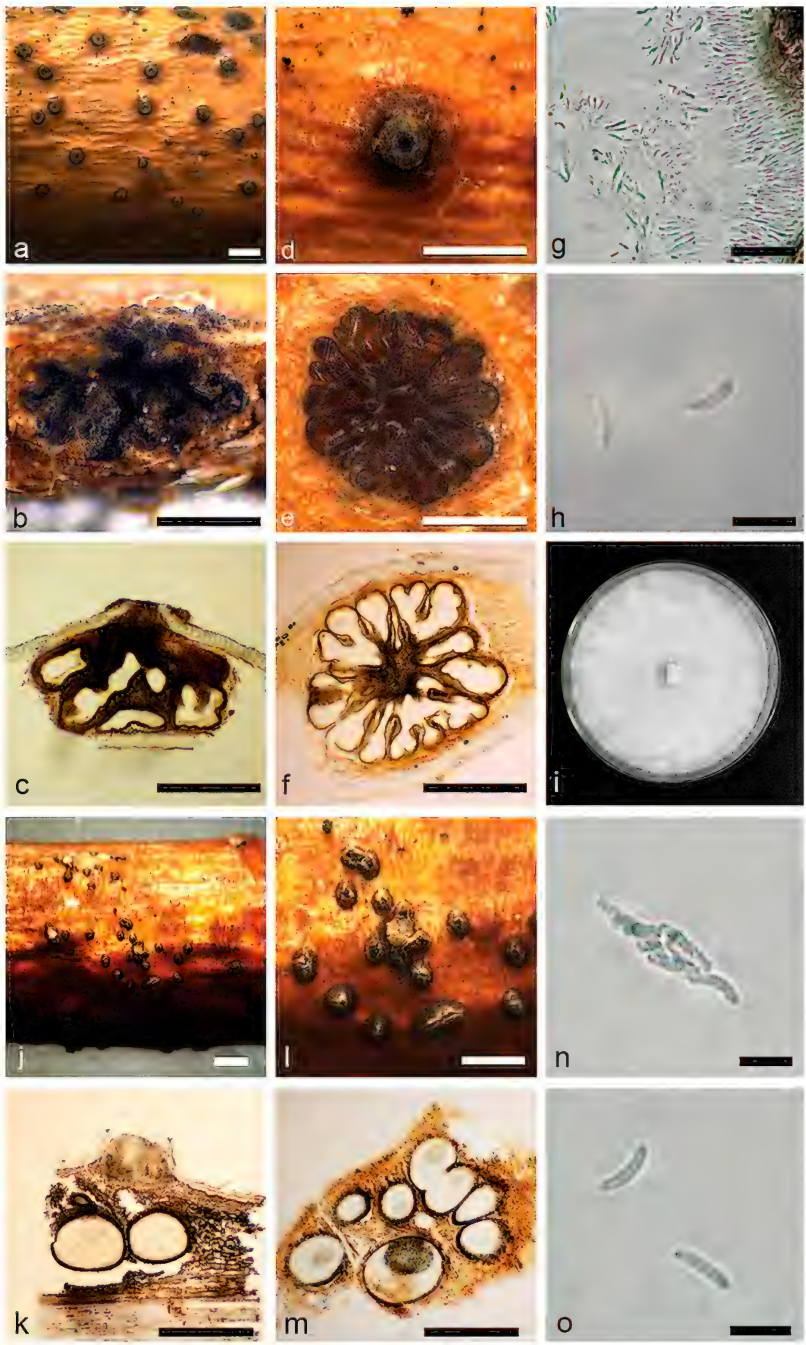
Teleomorph not found.

Additional specimens examined: **CHINA**, **Gansu Province**, Diebu, 34°04′48.35″N 103°23′36.60″E, elev. 2973 m asl, on stems of *Salix cupularis*, 9 August 2012, Y.M. Liang & X.L. Fan (BJFC-S727; culture BJFC-CGLs252; GenBank KC313891); Diebu, 34°04′44.65″N 103°23′39.42″E, elev. 2977 m asl, on stems of *Salix cupularis*, 9 August 2012, Y.M. Liang & X.L. Fan (BJFU-S733; culture BJFC-CGLs280, GenBank KC313892).

Cytospora chrysosperma (Pers.) Fr. Syst. Mycol. 2: 542, 1823. Plate 3

Conidiomata stromata immersed in bark, erumpent, discoid, flask-shaped to conical, with a large multiple locules. Disc grey to black, nearly flat, circular to ovoid, 0.21–0.33 mm (x = 0.22 mm, n = 20) in diameter, with one ostiole per disc. Ostiole medium grey, prominent, 62–85 µm (x = 76 µm, n = 20) in diameter. Locules complex multi-loculed, subdivided frequently by invaginations, sharing common walls, 0.64–1.26 mm (x = 1.01 mm, n = 20) in diameter. Conidiophores hyaline, unbranched or occasionally branched at the bases, 17.3–28.2 µm (x = 22.6 µm, n = 20). Conidia hyaline, eguttulate, elongate-allantoid, aseptate, 4.0–5.6 × 0.8–1.3 µm (x = 4.7× 1.2 µm, n = 50). Cultural characteristics were white originally, becoming partially pale yellow after 6–7 d although primarily white. The colony was flat, felt-like, and the texture was uniform. Conidiomata formed on PDA randomly.

Pl. 3 *Cytospora chrysosperma* (BJFC-CNLS2). **a** Conidiomata on twig. **b, c** Longitudinal sections through conidiomata. **d** Detail of beak on twig. **e, f** Transverse sections through conidiomata. **g** Conidiophores and conidiogenous cells. **h** Conidia. **i** Colonies on PDA at 3 d. **j, l** Habit of ascomata on twig. **k** Longitudinal sections through ascomata. **m** Transverse sections through ascomata. **n** Asci and immature ascospores. **o** Ascospores. Scale bars: a = 1 mm; b–f, j–m = 0.5 mm; g = 20 µm; h = 5 µm; n, o = 10 µm.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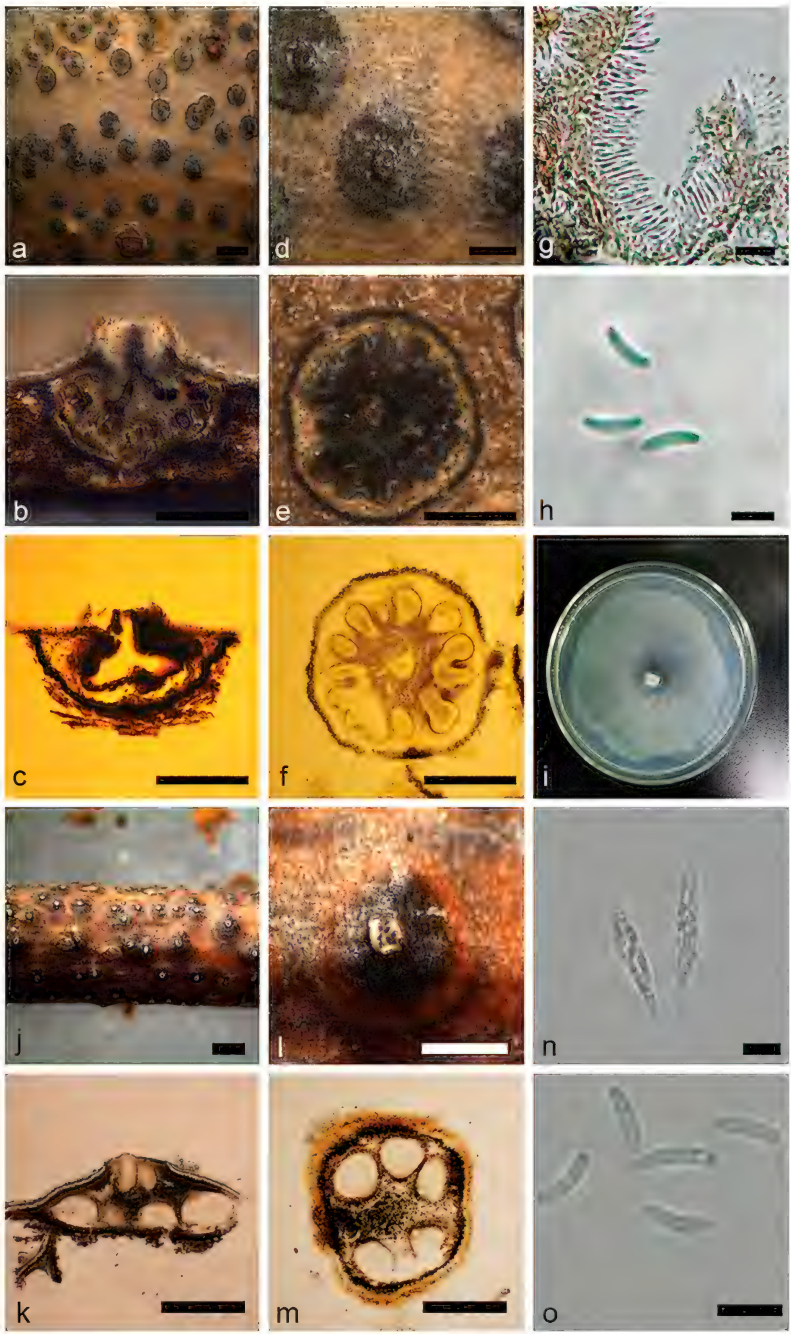
A huge ascostromatal region immersed in the bark, erumpent, extending basally up to 1.62–2.46 mm (x = 2.13 mm, n = 20) in diameter. Ascostromata usually scattered, erumpent, circular to ovoid, 0.86–1.19 mm (x = 0.97 mm, n=20) diameter. Disc was usually obscured by tightly ostiolar necks, when apparent pale brown to black, nearly hemispherical, circular to ovoid, 0.20–0.34 mm (x = 0.30 mm, n = 20) in diameter, with 4–8 ostioles arranged circinately in a disc, brown to black. 4–8 perithecia arranged circinately in black entostromata, flask-shaped to spherical, 0.26–0.34 mm (x = 0.30 mm, n = 20) in diameter. Asci free, clavate to elongate obovoid, 38.6–43.8 × 5.1–6.2 µm (x = 40.3 × 5.4 µm, n = 20), 8-spored. Ascospores, elongate-allantoid, thin-walled, hyaline, aseptate, 8.3–13.1 × 2.0–2.8 µm (x = 11.1 × 2.4 µm, n = 50). Cultural characteristics were white. The colony was flat, felt-like, and the texture was uniform. Conidiomata formed on PDA uniformly.

Materials examined: **China**, **Gansu Province**, Gannan, Lintan, Yeliguan, 34°57′36.48″N 103°39′55.12″E, elev. 2187 m asl, on stems of *Salix carmanica* Bornmüeller, 5 August 2012, X.L. Fan (BJFC-S702; culture BJFC-CGLs4; GenBank KC787359); Gannan, Zhuoni, Dayu National Forestry Park, 34°22′19.82″N 103°35′55.45″E, elev. 2181 m asl, on stems of *Salix cupularis*, 7 August 2012, X.L. Fan (BJFC-S706; culture BJFC-CGLs8; GenBank KC787360); Gannan, Lintan, Yeliguan, 34°57′43.41″N 103°39′44.82″E, elev. 2189 m asl, on stems of *Salix matsudana* Koidzumi, 5 August 2012, X.L. Fan (BJFC-S700; culture BJFC-CGLs2; GenBank KC787361); Gannan, Lintan, Yeliguan, 34°57′36.48″N 103°39′59.70″E, elev. 2187 m asl, on stems of *Salix matsudana* f. *tortuosa* (Vilm.) Rehder, 5 August 2012, X.L. Fan (BJFC-S705; culture BJFC-CGLs7; GenBank KC787362). **Ningxia Province**, Yinchuan, 38°31′51.80″N 106°17′48.05″E, elev. 1231 m asl, on stems of *Salix babylonica* L., 13 February 2012, X.L. Fan (BJFC-S508; culture BJFC-CNLs2; GenBank KC787357; culture BJFC-VNLs2; GenBank KC787365). **Shaanxi Province**, Weinan, Hua, Xiamiao, 34°34′47.66″N 109°45′41.61″E, elev. 335 m asl, on stems of *Salix babylonica* L., 26 May 2012, X.L. Fan (BJFC-S571; culture BJFC-CSLs3; GenBank KC787358).

Cytospora nivea (Hoffm.) Sacc., Michelia 2: 264, 1881, nom. illegit., non Fuckel 1860.
Plate 4

Conidiomata immersed in bark, erumpent, discoid, flask-shaped to conical, conceptacles dark, with a large multiple locules. Disc grey, nearly flat, became white when old, raised, circular to ovoid, 0.20–0.34 mm (x = 0.29 mm, n = 20) in diameter, with one ostiole per disc. Ostioles medium grey, prominent, 60–92 µm (x = 77 µm, n = 20) in diameter. Locules complex multi-loculed, subdivided frequently by invaginations, sharing common walls, 0.69–1.04 mm (x = 0.88

Pl. 4 *Cytospora nivea* (BJFC-CGLs32). **a** Conidiomata on twig. **b**, **c** Longitudinal sections through conidiomata. **d** Detail of beak on twig. **e**, **f** Transverse sections through conidiomata **g** Conidiophores and conidiogenous cells. **h** Conidia. **i** Colonies on PDA at 3 d. **j**, **l** Habit of ascomata on twig. **k** Longitudinal sections through ascomata. **m** Transverse sections through ascomata. **n** Asci and immature ascospores. **o** Ascospores. Scale bars: a, j = 1 mm; b–f, k–m = 0.5 mm; g = 20 µm; h = 5 µm; n, o = 10 µm.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mm, n = 20) in diameter. Conidiophores hyaline, unbranched or occasionally branched at the bases, 19.3–36.6 µm (x = 27.2 µm, n = 20). Conidia hyaline, eguttulate, elongate-allantoid, aseptate, 6.3–8.0 × 1.1–1.4 µm (x = 7.1 × 1.2 µm, n = 50). Cultural characteristics were white originally, became dark brown and black after 14 d. The colony was flat, felt-like, and the texture was uniform. Conidiomata formed on PDA uniformly.

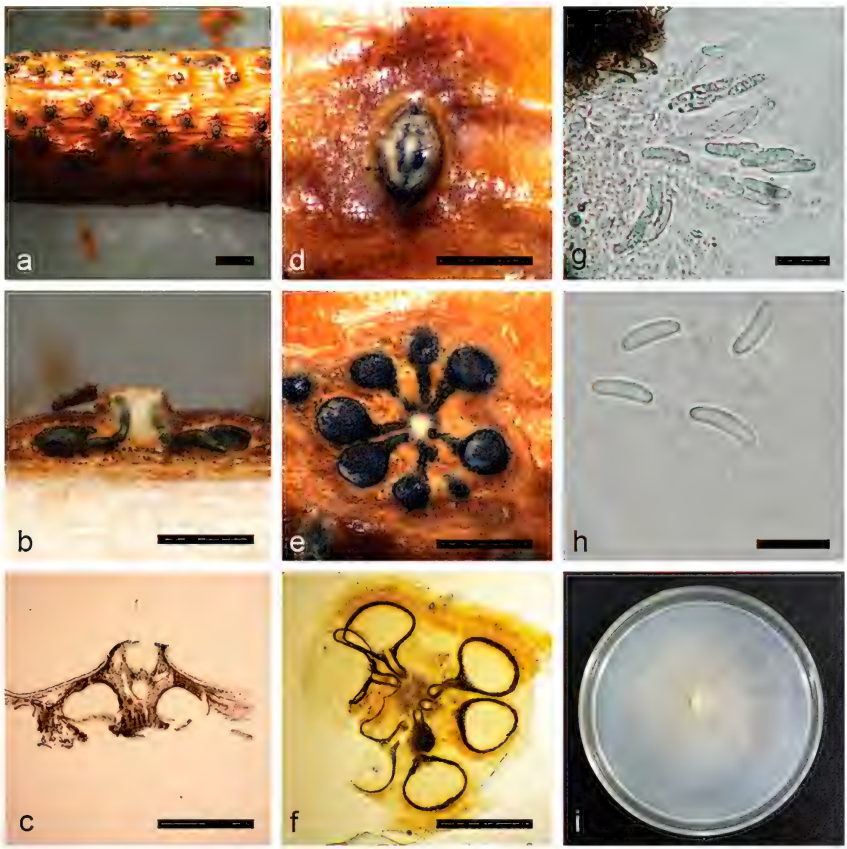
Ascostromata immersed in the bark, erumpent, circular to ovoid, 0.72–1.15 mm (x = 1.03 mm, n = 20) diameter, with a well defines black zone of demarcation, which was known as the conceptacle. Disc was usually obscured by tightly ostiolar necks, when apparent pale brown to beige, nearly flat, circular to ovoid, 0.19–0.27 mm (x = 0.24 mm, n = 20) in diameter, with 3–8 ostioles arranged circinately in a disc, brown to black. 3–8 perithecia arranged circinately in beige entostromata, flask-shaped to spherical, 0.23–0.31 mm (x = 0.28 mm, n = 20) in diameter. Asci free, clavate to elongate obovoid, 33.6–42.1 × 6.0–7.1 µm (x = 40.4 × 6.9, n = 20), 8-spored. Ascospores, elongate-allantoid, thin-walled, hyaline, aseptate, 10.7–14.8 × 2.4–2.9 µm (x = 12.9 × 2.6 µm, n = 50). Cultural characteristics were white originally, became dark brown and black after 14 d. The colony was flat, felt-like, and the texture was uniform.

Materials examined: **China**, **Gansu Province**, Gannan, Zhuoni, 34°35′16.64″N 103°09′39.53″E, elev. 2758 m asl, on stems of *Salix cupularis*, 8 August 2012, Y.M. Liang & X.L. Fan (BJFC-S730; culture BJFC-CGLs32; GenBank KC787364); Gannan, Zhuoni, 34°29′23.60″N 103°06′40.18″E, elev. 2540 m asl, on stems of *Salix cupularis*, 8 August 2012, Y.M. Liang & X.L. Fan (BJFC-S736; culture BJFC-VGLs33; GenBank KC787363).

Valsa populina (Pers.) Fuckel, Jahrb. Nassauischen Vereins Naturk. 25–26: 314, 1871.
Plate 5

Ascostromata immersed in the bark, erumpent, circular to ovoid, 0.90–1.27 mm (x = 1.07 mm, n = 20) diameter. Disc was usually obscured by tightly ostiolar necks, when apparent pale brown to beige, nearly flat, circular to ovoid, 0.19–0.24 mm (x = 0.22 mm, n = 20) in diameter, with 6–8 ostioles arranged circinately in a disc, brown to black. 6–8 perithecia arranged circinately in beige entostromata, flask-shaped to spherical, 0.25–0.26 mm in diameter. Asci free, clavate to elongate obovoid, 55.0–68.3 × 8.2–12.7 µm (x = 62.5 × 10.5 µm, n = 20), 4-spored. Ascospores, elongate-allantoid, thin-walled, hyaline, aseptate, 18.7–24.1 × 4.3–5.8 µm (x = 20.4 × 5.1 µm, n = 50). Cultural characteristics were white. The colony was flat, felt-like, and the texture was uniform. Conidiomata formed on PDA uniformly.

Material examined: **China**, **Gansu Province**, Gannan, Lintan, Yeliguan, 34°57′36.48″N 103°39′59.70″E, elev. 2181 m asl, on stems of *Salix matsudana* f. *tortuosa*, 5 August 2012, Y.M. Liang & X.L. Fan (BJFC-S716; culture BJFC-VGLs7; GenBank KC787366); Gannan, Pucigou, 34°04′51.38″N 103°23′34.33″E, elev. 3041 m asl, on stems of *Salix cupularis*, 9 August 2012, Y.M. Liang & X.L. Fan (BJFC-S725; culture BJFC-VGLs24; GenBank KC787367).



Pl. 5 *Valsa populina* (BJFC-VGLs7). **a** Ascomata on twig. **b, c** Longitudinal sections through ascomata. **d** Disc of ascomata on twig. **e, f** Transverse sections through ascomata **g** Asci and immature ascospores. **h** Ascospores. **i** Colonies on PDA at 3 d. Scale bars: a = 1 mm; b–f = 0.5 mm; g–h = 20 μm.

Discussion

Previously nine species of *Cytospora* and *Valsa* had been recorded on *Salix* in China. However, the identification of some of these species is questionable: the records of *C. capreae*, *C. salicis*, *C. fertilis*, *C. translucens*, *C. fugax*, and *C. germanica* share similar morphological characteristics but lack detailed descriptions and molecular analyses, and we agree that they should be unified under *C. fugax* (the name with priority) as suggested by Tai (1979). Consequently, based on earlier records only four species of *Cytospora* and *Valsa* from *Salix* can be accepted in northern China: *C. chrysosperma*, *C. fugax*, *C. ambiens*, and *V. populina*. In this study, we confirm the earlier reports of

C. chrysosperma and *V. populina* from *Salix* in China and add *C. nivea* as a new host record and *C. rostrata* as a new species.

Cytospora rostrata has a single locule without invaginations. *Cytospora pruinosa* (Fr.) Sacc., *C. eucalypticola* Van der Westh, *C. variostromatica* G.C. Adams & M.J. Wingf. also have single locules but differ from *C. rostrata* by having an ostiolar disc, rather than a thorn-like beak. All multi-locular species of *Cytospora* are further differentiated from *C. rostrata* by their lack of a thorn-like beak.

Phylogenetic analyses also support the proposal of *C. rostrata* as a new species. *Cytospora rostrata* has its closest genetic relationship with *C. chrysosperma*, which is the most widespread species of *Cytospora*.

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New record of *Melanoleuca cinereifolia* in Himalayan moist temperate forests of Pakistan

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Abstract — Basidiomata of *Melanoleuca cinereifolia* have been collected underneath *Pinus wallichiana* trees in Himalayan moist temperate forests of Pakistan. Its identification was confirmed by nrDNA ITS sequence analysis. A detailed morphological description and illustrations are provided. This is the first report of *M. cinereifolia* from Asia.

Key words —Khyber Pakhtoonkhaw, pine forest, saprobic fungi, *Tricholomataceae*

Introduction

The Himalayan moist temperate forests of Pakistan, located at elevations of 1373–3050 m, are characterized by vigorous thick vegetation of conifers and some deciduous trees. These mixed forests have an average rainfall of 59.3 cm with humidity up to 57% (Champion et al. 1968). Although the Himalayas are considered one of twenty-five biodiversity hotspots, most mushroom species are yet to be reported (Myers et al. 2000; Razaq et al. 2013). During research on saprobic fungi from pine-dominated forests of Western Himalayas, *Melanoleuca cinereifolia* was identified as a new record for Pakistan. *Melanoleuca* Pat. is a cosmopolitan genus characterized by collybioid to tricholomatoid basidiomata, convex to slightly depressed pilei (often with a shallow umbo), emarginate to adnate to shortly decurrent lamellae, absence of a veil, white to pale-yellowish spore print, cutis to trichoderm pileipellis, hyaline spores with amyloid ornamentations, and absence of clamp connections (Singer 1986, Boekhout 1988, Vizzini et al. 2012).

Singer (1935, 1943, 1986), Kühner (1978), Bon (1978), and Boekhout (1988) gave much importance to morphology (e.g., pileus, stipe and lamellar colour, and stipe ornamentation) and anatomy (cystidial shape and the basidiospore Q value) for infrageneric classification in *Melanoleuca*. Vizzini et al. (2012) used ITS sequence analyses to check the traditional tripartite *Melanoleuca* subgeneric classification (*M.* subg. *Acystis* without cystidia; *M.* subg. *Urticocystis* with

urticiform cystidia; *M.* subg. *Melanoleuca* with macrocystidia). He concluded that *Melanoleuca* is a monophyletic genus in which only two emended subgenera, *Urticocystis* and *Melanoleuca*, are supported; species previously placed in *M.* subg. *Acystis,* shown to be polyphyletic and no longer accepted, are now supported in *M.* subg. *Urticocystis*.

Two other *Melanoleuca* species — *M. exscissa* (Fr.) Singer and *M. angelesiana* A.H. Sm. — have previously been reported from Pakistan (Ahmad 1980, Razaq 2013).

Materials & methods

Collection and morphological examination

The basidiome (MSM#005) was collected, photographed, vouchered, dried under fan heater, and characterized morphologically. Specimen sections were mounted in 5% KOH for examination under a MX4300H biological microscope (Meiji Techno Co., Ltd., Japan); phloxine was used to increase contrast, and Melzer's reagent was used to test for amyloidity of the basidiospores.

Measurements of anatomical features (basidiospores, basidia, cystidia, pileus hyphae and stipe hyphae) were calculated from at least 20 measurements made with an ocular micrometer and 100× oil-immersion objective; abbreviations include x = mean spore length and width for all spores measured, Q = spore length / width ratio. Line drawings were made with a Lucida camera. Color designations are from Munsell (1975).

DNA extraction, PCR amplification, DNA sequencing

Genomic DNA was extracted from a small piece of pileus by a modified CTAB method (Bruns 1995). The internal transcribed spacers (ITS1, ITS2) + 5.8S region of the nuclear ribosomal RNA gene were targeted by the ITS1F/ITS4 primer pair (White et al. 1990; Gardes & Bruns 1993) using an Extract-N-Amp plant DNA extraction Kit (Sigma-Aldrich, St. Louis, MO, USA). The PCR amplification parameters were: initial denaturation (94 °C for 1 min), 35 cycles (94 °C for 1 min, 53 °C for 1 min, and 72 °C for 1 min), and final extension 72 °C (8 min). Amplified PCR products were sent for purification and bidirectional sequencing to Macrogen (Korea).

Sequence alignment and phylogenetic analysis

The nrITS sequence from MSM#005 comprised 740 base pairs. Initial blast analysis of nucleotide sequences revealed that the specimen showed 99% maximum identity with *Melanoleuca* aff. *cinereifolia*, *M. cinereifolia*, *M. communis* (GenBank JX429210, JX429108, JX429197, JX429207). GenBank sequences were selected based on the phylogenetic studies on *Melanoleuca* by Vizzini et al. (2012) and Sánchez-García et al. (2013).

Sequences were manually edited and assembled using BioEdit (www.mbio.ncsu.edu/bioedit/bioedit.html). Following Dentinger et al. (2011) for complete ITS sequences, all sequences were trimmed with the conserved motifs 5′-(...GAT)CATTA– and –GACCT(CAAA...)-3′ and the alignment portion between them were included in the analysis. Gaps were treated as data for construction of phylogeny.

The sequence from MSM#005 was aligned with GenBank sequences of *M. cinereifolia* and other related taxa (Table 1) by Muscle using the default setting in Molecular

Evolutionary Genetics Analysis (MEGA) software (Tamura et al. 2011). A phylogenetic tree was constructed with the Maximum Likelihood (ML) algorithm and Neighbor-Joining method, Jukes & Cantor (1969) model of nrITS sequences, and nearest-neighbor-interchange (NNI) as ML heuristic search method using MEGA5 software (Tamura et al. 2011). Phylogeny was tested by a bootstrap value of 1000 replicates, and bootstrap values >50% are cited in the tree.

Sequence of *M. cinereifolia* generated for this study was submitted to the Genbank and the accession numbers for this, as well as for other closely related taxa used in the phylogenetic analysis are cited in phylogenetic tree. Percent Identities (PID) and DNA divergence were calculated by DNAStar.

Table 1. *Limnoperdon* and *Melanoleuca* ITS-rDNA sequences used in the phylogenetic analysis. New sequence indicated in bold.

Taxon	Collection/Voucher	Country	Genbank #
L. incarnatum	IF030398	Japan	DQ097363
M. angelesiana	ANC M0203	Italy	JN616420
	ANC M0203	Italy	JN616421
M. brevipes	4574	Italy	JF908352
M. aff. *cinereifolia*	FCME11225	Mexico	JX429108
	IBUGJS89	Mexico	JX429210
M. cinereifolia	319	Italy	JN052137
	1471	Italy	JN052138
	MSM#005	**Pakistan**	**KJ182965**
M. cognata	13939	Italy	JF908360
	ANC M0170	Italy	JN616425
M. communis	XAL Murrieta1025	Mexico	JX429204
	FCME 17116	Mexico	JX429207
	ENCB Guzman 6329	Mexico	JX429226
M. decembris	ANC M0197	Italy	JN616426
	ANC M0200	Italy	JN616428
M. exscissa	ANC M0210	Italy	JN616433
	ANC M0213	Italy	JN616436
M. grammopodia	ANC M0217	Italy	JN616439
	ANC M0218	Italy	JN616440
M. heterocystidiosa	ANC M0174	Italy	JN616444
	ANC M0175	Italy	JN616445
M. nivea	MCVE 9578	Italy	JN392452
	ANC M0177	Italy	JN616450
M. paedida	ANC M0189	Italy	JN616452
	ANC M0190	Italy	JN616453
M. pseudoluscina	ANC M0194	Italy	JN616458
	ANC M0195	Italy	JN616459
M. strictipes	ANC M0172	Italy	JN616465
	ANC M0173	Italy	JN616466
M. subpulverulenta	ANC M0004	Italy	JN616472
	ANC M0178	Italy	JN616473
M. substrictipes	ANC M0214	Italy	JN616474

Fig. 1. *Melanoleuca cinereifolia* (MSM#005). Basidiomes. Scale bars: 10 mm.

Taxonomy

Melanoleuca cinereifolia (Bon) Bon, Docum. Mycol. 9(33): 71 (1978). Figs 1–3

Description based on a single basidiome:

Pileus 21 mm diam., plano-convex, flat, thin (collybioid); margin straight or flaring, smooth; surface dull, off-white to grayish near margin (5R9/2), central disc chocolate brown (5YR1/2). Lamellae free or approximate, close, off-white to gray (5YR9/2). Stipe 26 mm, central, subequal, often with subbulbous base, hollow, fibrillose, light brown (5YR7/4). Rhizomorphs white, few. Odor and Taste not recorded. Basidiospores 4.3–6.1 × 7.2–9.5 µm [x = 5.4 × 8.5 µm, Q = 1.57], oblong or ellipsoid, apiculus absent, warty, thin-walled, hyaline in KOH, amyloid. Basidia 8.5–11.8(–13) × 28.7–38.2(–44.8) µm, clavate, mono-, bi-, or tetra-spored, thin-walled, hyaline in KOH; sterigmata 1.8–4.2(–5.1) µm. Trama hyphal, 6.1–8.9 µm, thin-walled, hyaline in KOH. Cheilocystidia 11.8–14(–16.9) × 47–71(–77.4) µm, lageniform with crystals at the apex, sometimes septate, hyaline, slightly thick walled, up to 1 µm. Pleurocystidia similar to cheilocystidia. Pileipellis a cutis, hyphae cylindrical, 9–16 µm, thin-walled, hyaline in KOH. Stipe Hyphae cylindrical, 5–11.8(–14.8) µm, hyaline to pale yellow in KOH. Clamp connections absent.

Material examined: **PAKISTAN**, **Khyber Pakhtoonkhaw**, Shangla, Yakh Tangay, under *Pinus wallichiana* A.B. Jacks., 2 September 2013, coll. M. Saba & A.N. Khalid, MSM#005 (LAH; GenBank KJ182965).

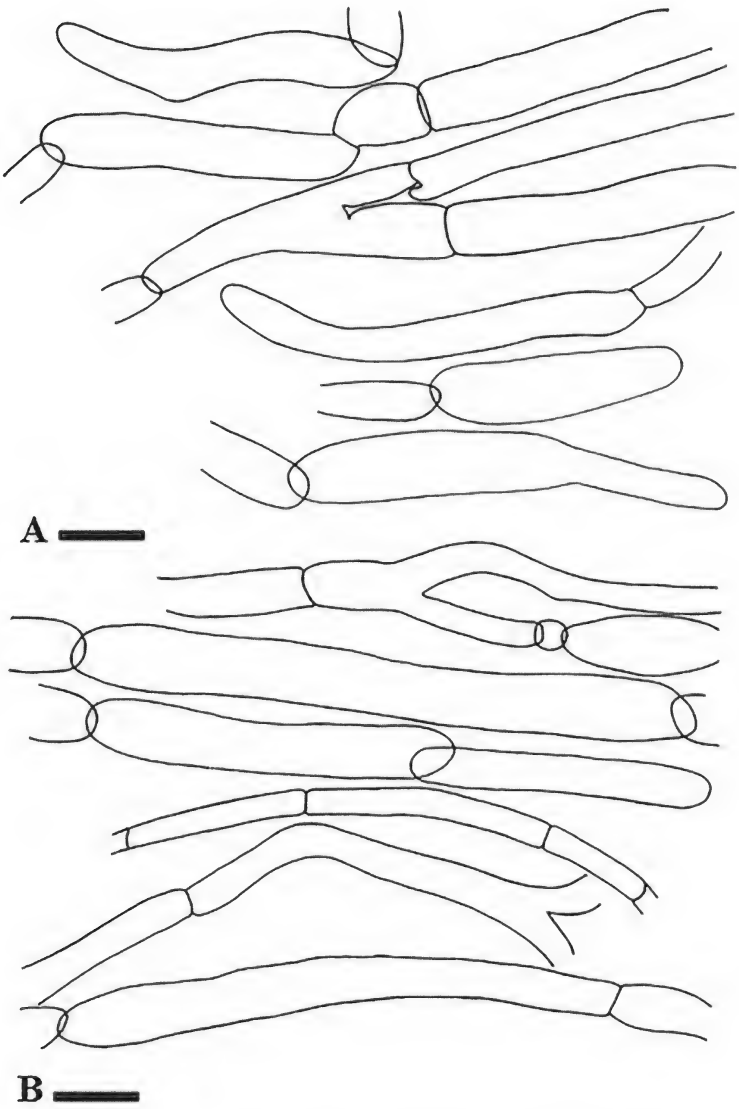

Fig. 2. *Melanoleuca cinereifolia* (MSM#005). A, pileipellis; B, stipitipellis. Scale bars: A = 20 µm, B = 16 µm.

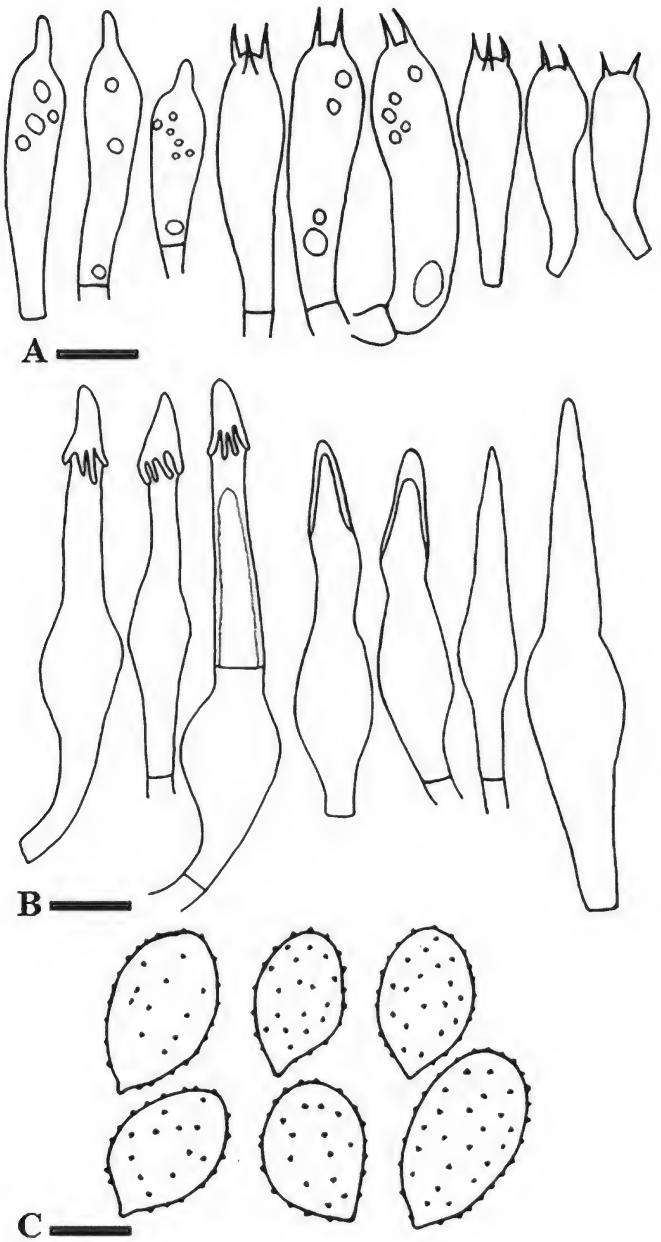

Fig. 3. *Melanoleuca cinereifolia* (MSM#005). A, basidia; B, cheilocystidia; C, basidiospores. Scale bars: A, B = 10 µm; C = 3.6 µm.

Results

The phylogenetic trees were constructed with the Maximum Likelihood algorithm (Fig. 4) and Neighbour-Joining method (Fig. 5), using Jukes & Cantor (1969) model of nrITS sequences and nearest-neighbor-interchange (NNI) as ML heuristic search method using MEGA5 software (Tamura et al. 2011). Codon positions included were 1st+2nd+3rd+Noncoding. The

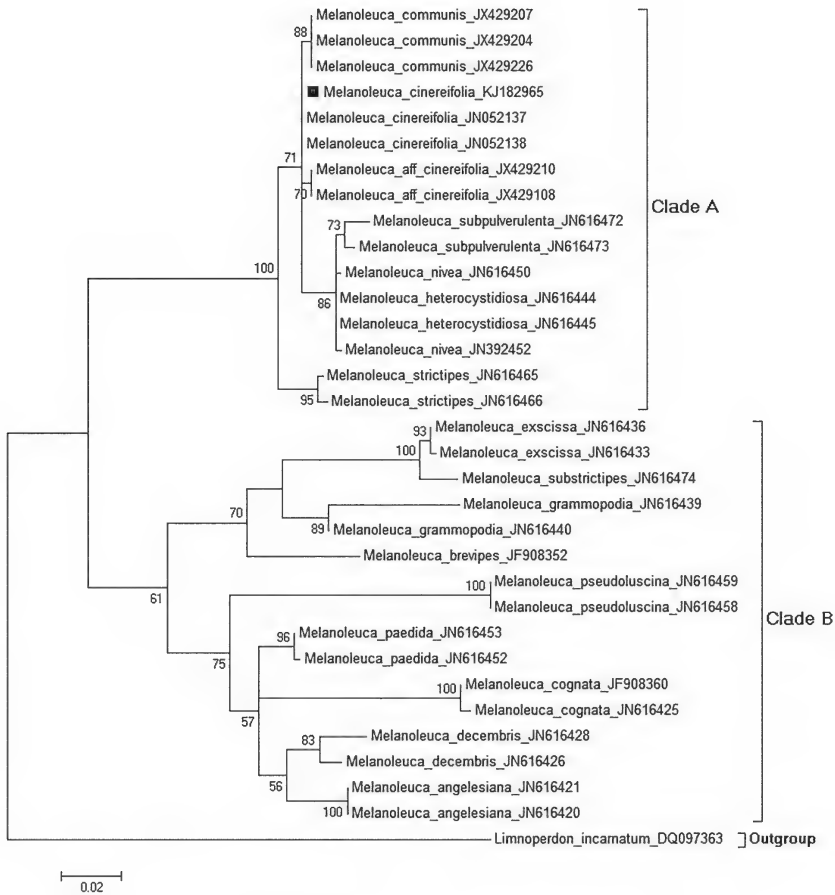

Fig. 4. Phylogenetic relationship of *Melanoleuca cinereifolia* with other *Melanoleuca* spp. based on Maximum Likelihood method inferred from nrITS sequences. The tree with the highest log likelihood (–3381.2856) is shown. Bootstrap values >50 (1000 replicates) are shown below the branches, and the percentage of trees in which the associated taxa cluster together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

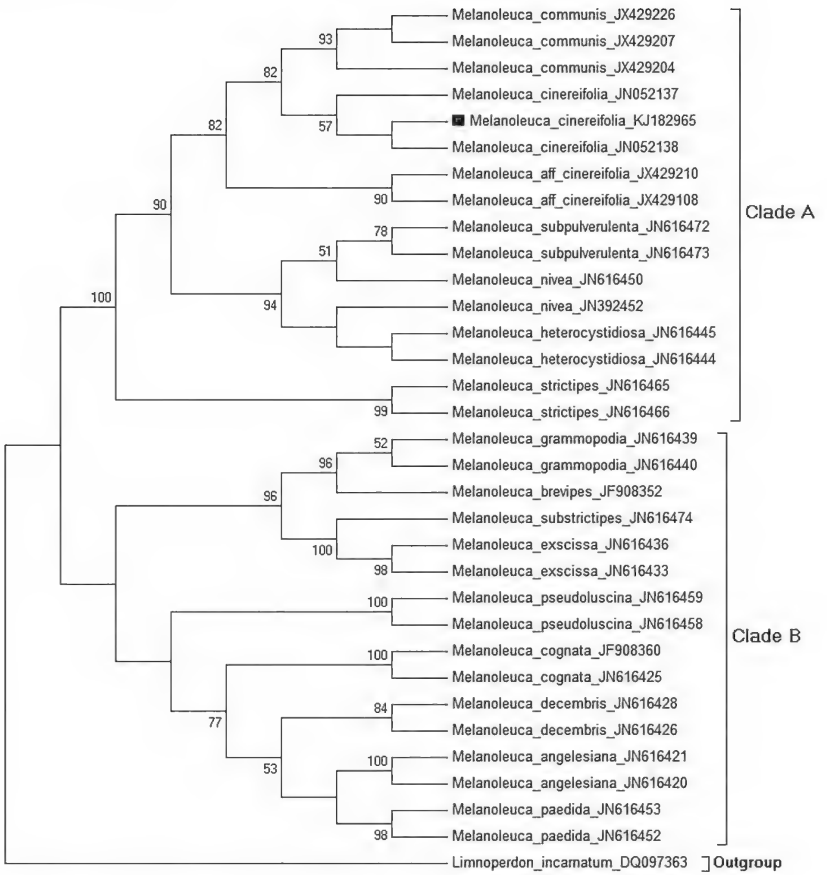

FIG. 5. Phylogenetic relationship of *Melanoleuca cinereifolia* with other *Melanoleuca* spp. based on Neighbor-Joining method. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. Evolutionary distances were computed using the Jukes-Cantor method and are in the units of the number of base substitutions per site.

final dataset contains 33 nucleotide sequences. *Limnoperdon incarnatum* (DQ097363) was used as outgroup based on results from Vizzini et al. (2012).

After removing and editing the ambiguous letters from aligned datasheet, a total of 711 characters were subjected for phylogenetic analysis, of which 435 characters were conserved, 274 were variable, 186 were parsimony informative, and 87 were singletons.

The percentage similarity was calculated by MegAlign (DNA Star Inc.). *Melanoleuca cinereifolia* showed 99.4% identity and 0% genetic divergence with sequences retrieved from GenBank (JN052137, JN052138), 99.4% identity and 0.5% divergence with *M.* aff. *cinereifolia* sequences (JX429108, JX429210), and 99.3% identity and 0.5% divergence with *M. communis* (JX429226, JX429204, JX429207).

Discussion

Melanoleuca cinereifolia and *M. communis* M. Sánchez-García & J. Cifuentes closely resemble each other. Both species show 99% maximum similarity to the present species in the Blast search and probably represent the same taxon. In the phylogeny MSM#005 clusters with *M. cinereifolia* and morphologically, it differs from *M. communis* in basidiome and pileus size and pileus and lamellar colour. *Melanoleuca cinereifolia* is usually characterized as a basidiome with a short stipe, grey lamellae, lageniform cystidia that grows in sand dunes (Vizzini et al. 2012). Lantieri et al. (2009), describe it from Italy as frequent on sand dunes characterized by *Cypero capitati-Agropyretum juncei* and as well as at higher elevations characterized by *Medicagini marinae-Ammophiletum australis*. In Pakistan, our *M. cinereifolia* specimen was collected growing on forest floor rich in organic matter in a pure *Pinus wallichiana* forest. Sánchez-García et al. (2013) collected *M.* cf. *cinereifolia* in mountainous mesophilic forests in Mexico.

Other *Melanoleuca* species known from Pakistan are *M. exscissa* and *M. angelesiana* (Ahmad 1980; Razaq 2013). Unlike *M. cinereifolia, M. exscissa* has a urticiform *exscissa* cystidial type while *M. angelesiana* lacks cystidia.

Identification of *Melanoleuca* species is difficult because most morphological characters are homoplastic and influenced by environmental factors (Vizzini et al. 2012), making molecular analysis necessary for species identification.

Our phylogenetic trees (Figs 4, 5) divide *Melanoleuca* into two major clades, supporting recognition of two subgenera (*Melanoleuca* and *Urticocystis*). Clade A in which the Pakistani specimen is nested is supported by a robust (100) bootstrap and includes taxa mainly with lageniform or fusiform macrocystidia. Clade B, which is supported by only 56 bootstrap, includes all taxa with urticoid cystidia, some species lacking cystidia, and the macrocystidiate *M. cognata* complex. *Melanoleuca exscissa* and *M. angelesiana* reported from Pakistan cluster in clade B. Our phylogeny agrees closely with the results reported by Vizzini et al. (2012). Moreover, percent identity and genetic divergence support the identity of MSM#005 as *M. cinereifolia*, as inferred from the phylogenetic tree.

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Entoloma species from New South Wales and northeastern Queensland, Australia

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Abstract — Seven new species in the Prunuloides clade of the *Entolomataceae* are described here: *Entoloma hymenidermum* is diagnosed by blackish blue basidiomata, isodiametric basidiospores and moderately broad pileocystidia; *E. violaceotinctum* has a violet-tinged pileus, violaceous-tinged stipe, and broad inflated pileocystidia; *E. discoloratum* possesses a subviscid yellow-tinged white pileus; *E. kewarra* is distinguished by its yellow pileus and stipe, both with a white and then eventually greenish yellow context; *E. pamelae* has a smooth, bright yellow, dry pileus; *E. rugosiviscosum* has a yellow-brown, rugose viscid pileus; and *E. guttulatum* is distinguished by lamellae with droplets that become reddish brown on drying.

Key words — *Basidiomycota*, phylogeny, taxonomy

Introduction

A morphologically based classification has given rise to two interpretations of the genus *Entoloma* (*Agaricales*, *Entolomataceae*). In one approach, taxa are placed into a single genus and the subgenera are defined mostly by the pileus surface, the pileipellis structure, and the basidiospore size, shape, and angularity (Noordeloos 1992, 2005; Co-David et al. 2009; Noordeloos & Gates 2012). In a second approach, *Entoloma* is accepted in a restricted sense with additional genera recognized based on biochemical features, pigmentation, location of clamp connections, and stature types in addition to the pileus surface, the pileipellis structure, and the basidiospore size, shape, and angularity (Orton 1991, Largent 1994, Baroni & Matheny 2011, Baroni et al. 2011). Recent comprehensive phylogenetic analyses of the *Entolomataceae* have highlighted

many issues inherent with the morphologically based classifications by showing that many of the segregate genera or infrageneric groups are not monophyletic (Co-David et al. 2009; Baroni & Matheny 2011), suggesting the need to re-evaluate key characters used for delineation of segregate genera.

The majority of species of *Entoloma* s.s. are supported in one of two clades, either the Rhodopolioid or Prunuloides clade (Co-David et al. 2009, Baroni & Matheny 2011). The species described in this paper share similar features with many members of the Prunuloides clade: a tricholomatoid or naucorioid stature (Largent 1986), isodiametric basidiospores, rather short and broad hyphae in the pileus trama, and abundant clamp connections in the pileipellis.

The Prunuloides clade also accommodates segregate genera. *Entocybe* T.J. Baroni et al. was erected for species with finely pustulate basidiospores with bumps and 6–10 facets when viewed in polar view (Baroni et al. 2011). *Calliderma* (Romagn.) Largent was erected for species with a tricholomatoid stature, a pruinose, tomentose, velutinous, or rivulose pileus corresponding to a hymeniform pileipellis, and septate hyphae with or without clamp connections.

Australian field investigations from 2009–2012 within northeastern Queensland's Wet Tropics Bioregion and from 2010–2012 in the temperate rainforests of central New South Wales have uncovered several novel entolomataceous species (Largent & Abell-Davis 2011; Largent et al. 2011a,b, 2013a,b; Bergemann et al. 2013). Here, we describe seven new species representing *Entoloma* s.s.: *E. discoloratum*, *E. guttulatum, E. hymenidermum*, *E. kewarra*, *E. pamelae*, *E. rugosiviscosum*, and *E. violaceotinctum*.

Materials & methods

Macromorphological and micromorphological features

Techniques and equipment for collecting and describing basidiomata in the field, GPS coordinates, microscopy of dried collections including how basidiospores were measured, and digital microphotographs have been described in Largent et al. (2011a, b), while techniques for color descriptions using Kornerup & Wanscher (1978) and factors determined from mathematical analyses in the descriptions are covered in Largent et al. (2013a,b). All collections for New South Wales cited in the 'Additional collections examined' were deposited in The Plant Pathology Herbarium, Orange Agricultural Institute (DAR). Collections made in Queensland were deposited in the Australian Tropical Herbarium (CNS). All holotype and isotype collections are deposited in the herbaria designated using acronyms from Thiers (2012).

DNA sequences and phylogenetic analyses

Thirty-four sequences from three partial genes including the mitochondrial small subunit ribosomal RNA (mtSSU), the nuclear large subunit ribosomal RNA (LSU), and second largest subunit of RNA polymerase II (RPB2) were generated for this phylogenetic analysis along with sequences obtained from GenBank (Table 1). The extraction and Polymerase Chain Reaction (PCR) protocols for the mtSSU, LSU and the RPB2 follow

TABLE 1. Collections used in the phylogenetic analyses. New sequences generated for this study are shown in bold. Square bracketed annotations indicate species names applied in GenBank that differ from those in the phylogram (FIG. 1).

SPECIES FROM GENBANK	COLLECTION IDENTIFIER	GENBANK ACCESSION NUMBERS		
		mtSSU	LSU	RPB2
Entocybe haastii	DLL9868	JQ793644	JQ793651	JQ793658
	DLL10087	JQ793645	JQ793652	JQ793659
Ec. nitida [*E. nitidum*]	7526 TJB	GU384602	GU384626	GU384655
Ec. nitida [*E. alcedicolor*]	210	GQ289292	GQ289152	GQ289224
Ec. trachyospora [*R. trachyospora*]	5856 TJB	GU384605	GU384629	GU384658
Ec. turbida [*E. turbidum*]	27	GQ289341	GQ289201	GQ289269
	6949 TJB	GQ289341	GQ289201	GQ289269
Ec. vinacea [*E. vinaceum*]	8870 TJB	GU384598	GU384631	GU384651
Clitopilus* aff. *hobsonii	**DLL9586**	**KJ021688**	**KJ021698**	KC816912
Entoloma albomagnum	427	KC710165	KC710137	—
E. bloxamii	219	GQ289294	GQ289154	GQ289226
E. aff. *bloxamii*	628	KC710189	KC710159	—
	Thiers 53901	KC710168	KC710139	—
E. aff. *whiteae* [*E. prunuloides*]	4765 TJB	—	AY700180	DQ385883
Entoloma sp. [*E. haastii*]	BY21	—	AF261309	—
Entoloma sp.	292, 9895 TJB	GQ289296	GQ289156	GQ289228
E. caccabus	17	GQ289295	GQ289155	GQ289227
E. caesiolamellatum [*E. bloxamii*]	6117 TJB	—	AF261289	—
E. callidermum	609	KC710183	KC710153	—
E. calongei	322	GQ289298	GQ289158	—
E. clypeatum	41	KC710164	KC710136	—
E. coeruleogracilis [*E. haastii*]	216	GQ289308	GQ289168	GQ289239
	217	GQ289309	GQ289169	GQ289240
E. coeruleoviride	609	KC710162	KC710134	KC710057
E. cretaceum	213	GQ289302	GQ289162	GQ289233
E. discoloratum	**DLL10217**	**JQ793646**	**JQ793653**	**JQ793660**
E. ferruginans	**11CA032**	**KJ021689**	**KJ021699**	**KJ021693**
E. fibulatum [*Calliderma fibulatum*]	SP393751	—	FJ973677	—
E. flavifolium	6215 TJB	GU384597	AF261301	GU384644
	621	KC710179	KC710150	—
E. fragilum	**MCA2415**	**KJ021690**	**KJ021700**	**KJ021694**
E. fumosobrunneum [*Entoloma* sp.]	2005113	KC710185	KC710155	—
	2005120	KC710186	KC710156	—
E. gelatinosum	212	GQ289305	GQ289165	GQ289236
E. griseolazulinum	i11	GQ289306	GQ289166	GQ289237
E. guttulatum	**DLL9791**	—	—	**JQ793656**
E. hymenidermum	**DLL10025**	**JQ793648**	**JQ793642**	—
	DLL10054	—	**JQ793649**	—
E. illinitum	**MCA2488**	—	**KJ021701**	**KJ021695**
E. indigoticoumbrinum	83	GQ289318	GQ289178	GQ289249
E. kermandii	222	GQ289313	GQ289173	GQ289244
E. kewarra	**DLL10055**	—	**JQ793655**	**JQ793662**
E. lividoalbum	233	KC710182	KC710152	—

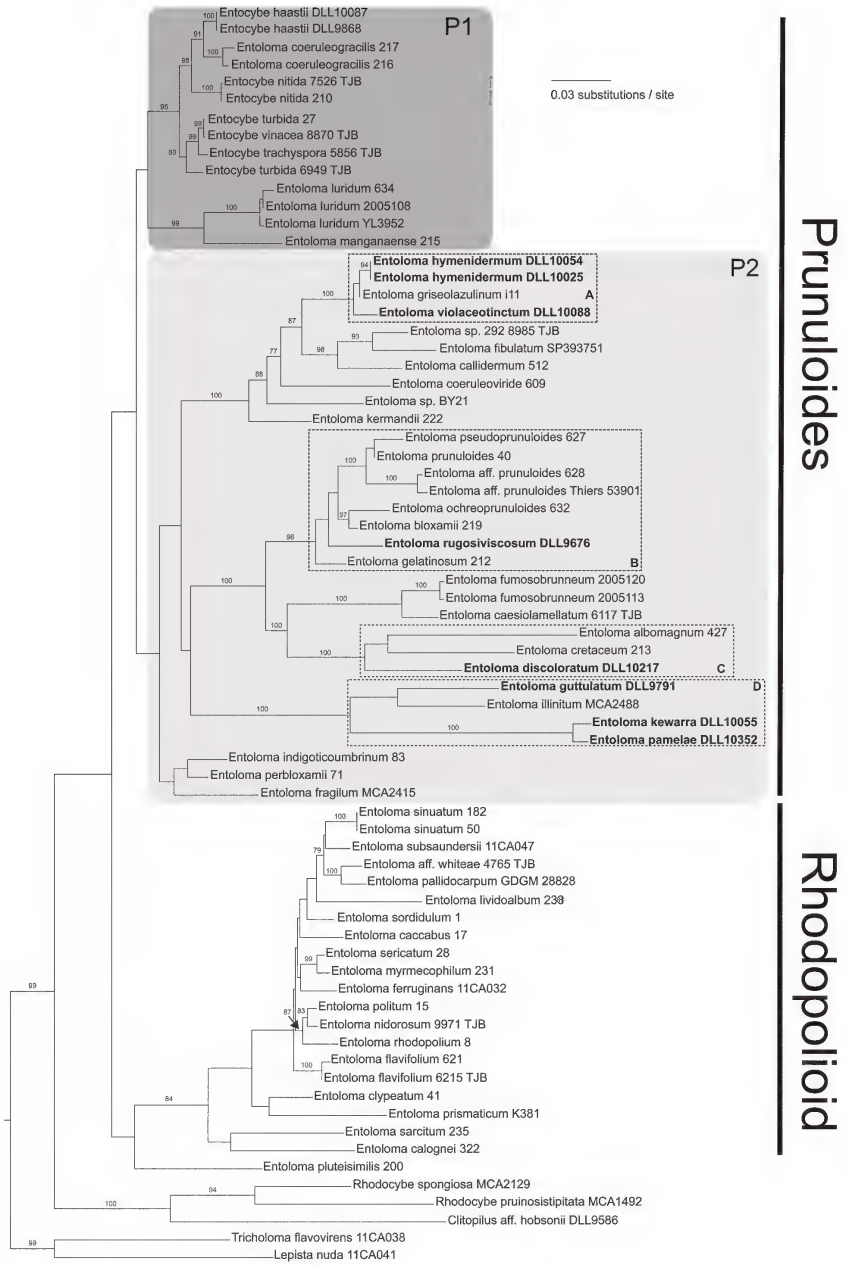
Table 1, concluded

Species from GenBank	Collection Identifier	GenBank Accession Numbers		
		mtSSU	LSU	RPB2
E. luridum	2005108	KC710175	KC710146	KC710192
	634	KC710170	KC710141	—
	YL3952	KC710177	KC710148	—
E. manganaense	215	KC710172	KC710143	—
E. myrmecophilum	231	GQ289314	GQ289174	GQ289245
E. nidorosum	9971 TJB	GU384596	GU384617	GU384643
E. ochreoprunuloides [*Entoloma* sp.]	632	KC710176	KC710147	—
E. pallidocarpum	GDGM 28828	JQ993074	JQ410331	JQ993080
E. pamelae	**DLL10352**	—	**KJ021702**	**KJ021696**
E. perbloxamii	71	GQ289318	GQ289178	GQ289249
E. pluteisimilis	200	GQ289320	GQ289180	GQ289251
E. politum	15	GQ289321	GQ289181	GQ289252
E. prismaticum	K381	—	AB692006	AB692016
E. prunuloides	40	GQ289324	GQ289184	GQ289255
E. pseudoprunuloides [*Entoloma* sp.]	627	KC710169	KC710140	—
E. rhodopolium	8	GQ289327	GQ289187	GQ289258
E. rugosiviscosum	**DLL9676**	**JQ793647**	**JQ793654**	**JQ793661**
E. sarcitum	235	GQ289328	GQ289188	GQ289259
E. sericatum	28	GQ289329	GQ289189	—
E. sinuatum	50	GQ289333	GQ289193	GQ289264
	182	KC710184	KC710154	—
E. sordidulum	1	GQ289334	GQ289194	GQ289265
E. subsaundersii	**11CA047**	—	**KJ021703**	**KJ021697**
E. violaceotinctum	**DLL10088**	**JQ793643**	**JQ793650**	**JQ793657**
Lepista nuda	**11CA041**	**KJ021692**	**KJ021705**	**KJ136110**
Rhodocybe pruinosistipitata	MCA1492	GU384608	GU384627	GU384653
R. spongiosa	MCA2129	GU384604	GU384628	GU384657
Tricholoma flavovirens	**11CA038**	**KJ021691**	**KJ021704**	KC816997

Largent et al. (2011b). The primer combinations rpb2-i6f and rpb2-i7r (Co-David et al. 2009), rpb2-EntF2 and rpb2-EntR4 (Largent et al. 2013b) and fRPB2-5F and bRPB2-7R (Liu et al. 1999; Matheny 2005) were also used. Sequences were generated on an Applied Biosystems 3130*xl* Genetic Analyzer at Middle Tennessee State University using the sequencing protocols outlined in Largent et al. 2011b.

Sequences were edited using Sequencher 4.2.2 (Gene Codes Corporation, Ann Arbor, MI) and an automated alignment performed using MAFFT v. 7 (Katoh & Standley 2013). Introns in the RPB2 and hypervariable regions and large introns in the mtSSU were excluded prior to analysis. The alignment lengths were 570 bp (mtSSU), 1506 bp (LSU), and 1165 bp (RPB2). A Maximum Likelihood analysis based on 100

Figure 1. Topology of the maximum-likelihood phylogram (mtSSU+LSU+RPB2) highlighting the subclades within the Prunuloides clade that included the new species proposed here (P1 and P2). Each sequence is labeled with the GenBank-listed isolate identifier and species placement for clusters (A–D) are described in the text. Branches with ≥70% support from 1000 rapid bootstraps are shown. *Lepista nuda, Clitopilus* aff. *hobsonii, Rhodocybe pruinosistipitata, R. spongiosa,* and *Tricholoma flavovirens*, served as outgroups, and the tree was rooted with *L. nuda*.


P1
P2
Prunuloides
Rhodopoloid
0.03 substitutions / site
Entocybe haastii DLL10087
Entocybe haastii DLL9868
Entoloma coeruleogracilis 217
Entoloma coeruleogracilis 216
Entocybe nitida 7526 TJB
Entocybe nitida 210
Entocybe turbida 27
Entocybe vinacea 8870 TJB
Entocybe trachyspora 5856 TJB
Entocybe turbida 6949 TJB
Entoloma luridum 634
Entoloma luridum 2005108
Entoloma luridum YL3952
Entoloma manganaense 215
Entoloma hymenidermum DLL10054
Entoloma hymenidermum DLL10025
Entoloma griseolazulinum i11
Entoloma violaceotinctum DLL10088
Entoloma sp. 292 8985 TJB
Entoloma fibulatum SP393751
Entoloma callidermum 512
Entoloma coeruleoviride 609
Entoloma sp. BY21
Entoloma kermandii 222
Entoloma pseudoprunuloides 627
Entoloma prunuloides 40
Entoloma aff. prunuloides 628
Entoloma aff. prunuloides Thiers 53901
Entoloma ochreoprunuloides 632
Entoloma bloxamii 219
Entoloma rugosiviscosum DLL9676
Entoloma gelatinosum 212
Entoloma fumosobrunneum 2005120
Entoloma fumosobrunneum 2005113
Entoloma caesiolamellatum 6117 TJB
Entoloma albomagnum 427
Entoloma cretaceum 213
Entoloma discoloratum DLL10217
Entoloma guttulatum DLL9791
Entoloma illinitum MCA2488
Entoloma kewarra DLL10055
Entoloma pamelae DLL10352
Entoloma indigoticoumbrinum 83
Entoloma perbloxamii 71
Entoloma fragilum MCA2415
Entoloma sinuatum 182
Entoloma sinuatum 50
Entoloma subsaundersii 11CA047
Entoloma aff. whiteae 4765 TJB
Entoloma pallidocarpum GDGM 28828
Entoloma lividoalbum 230
Entoloma sordidulum 1
Entoloma caccabus 17
Entoloma sericatum 28
Entoloma myrmecophilum 231
Entoloma ferruginans 11CA032
Entoloma politum 15
Entoloma nidorosum 9971 TJB
Entoloma rhodopolium 8
Entoloma flavifolium 621
Entoloma flavifolium 6215 TJB
Entoloma clypeatum 41
Entoloma prismaticum K381
Entoloma sarcitum 235
Entoloma calognei 322
Entoloma pluteisimilis 200
Rhodocybe spongiosa MCA2129
Rhodocybe pruinosistipitata MCA1492
Clitopilus aff. hobsonii DLL9586
Tricholoma flavovirens 11CA038
Lepista nuda 11CA041

ML replicates and specifying a GTRGAMMA model in RAxML-HPC v. 7.2.8 ALPHA (Stamatakis 2006) was carried out with a partitioned dataset: RPB2 (across each codon position for three partitions), and separate partitions for the mtSSU and LSU. Support values were based on bootstrap proportions on 1000 bootstrap replicates (Stamatakis et al. 2008).

Results

The phylogenetic analysis based on combined mtSSU+LSU+RPB2 genes place all seven *Entoloma* species into the Prunuloides clade (Fig. 1). Clade P1 contains a cluster of species recognized as *Entocybe* (BS = 98) and a sister clade including *E. luridum* Hesler and *E. manganaense* G.M. Gates & Noordel. (BS = 99), a relationship that remains unsupported here. Clade P2 contains all the Australian species described in this report: 1) *Entoloma hymenidermum* and *E. violaceotinctum* cluster with several *Calliderma* species and share a close affinity with *E. griseolazulinum* Manim. & Noordel. (BS = 100, Fig. 1, A); 2) *Entoloma rugosiviscosum* clusters with *E. bloxamii* (Berk. & Broome) Sacc., *E. gelatinosum* E. Horak, and species within the *E. prunuloides* species complex (BS = 98, Fig. 1, B), whereas *E. discoloratum* cluster with *E. cretaceum* G.M. Gates & Noordel. and *E. albomagnum* G.M. Gates & Noordel. (BS = 100, Fig. 1, C); and 3) *E. guttulatum*, *E. pamelae* and *E. kewarra* cluster together with *E. illinitum* Largent & Aime (BS = 100, Fig. 1, D).

Taxonomy

Entoloma hymenidermum Largent, **sp. nov.** Plates 1–2

MycoBank MB804379

Differs from *Entoloma griseolazulinum* by the broader stipe, smaller basidia, and shorter basidiospores.

Type — Australia, Queensland, Cook Region, Yorkeys Knob coastal vine forest, within 20m of 16°49′00.3″S 145°43′49.3″E, 20 March 2011, DL Largent 10054 (**holotype** BRI; **isotype** CNS).

Etymology — named for the hymeniderm pileipellis.

Pileus 29–70 mm broad, 6.5–15.0 mm high; opaque, not hygrophanous, not translucent-striate; at first convex to broadly convex, sometimes campanulate-convex, upon expansion and maturity becoming plane and finally uplifted and undulate, sometimes broadly umbonate; entirely minutely velvety to suede-like but forming irregularly radial cracks near the margin when mature, always dull; when young blackish blue with the bluish color dominant (20F8) in the center, slightly lighter (20F6) elsewhere, on drying dark blue (20E-F6), when old blackish blue with the black color dominating (19F4); margin incurved at first, then decurved, finally plane then uplifted, entire then eroded; context 1–3 mm thick above the stipe, bluish. Odor mild, at times somewhat fragrant.

Plate 1. *Entoloma hymenidermum* (DLL10054 holotype). A. Basidiomata. B. Stipe surface. C. Pileus surface. Bar scale: A = 15 mm; B = 5 mm; C = 8 mm.

Taste mild to at times somewhat bitter. Lamellae 11–29 mm long, 2.7–10.5 mm deep, narrow to moderately broad and eventually nearly broad; uncinate to sinuate with a decurrent tooth; close then subdistant; white to between white and orange white (5A1–2) when young becoming greyish orange (5A-B4–5) or (5A3–4) with basidiospore maturation; margin smooth and concolorous. Stipe 27–79 mm long, 4–10.5 mm broad at the apex, 4–14.5 mm broad at base, equal to clavate; longitudinally appressed fibrillose from the apex to the basal tomentum, markedly striate, and sometimes twisted; fibrils various

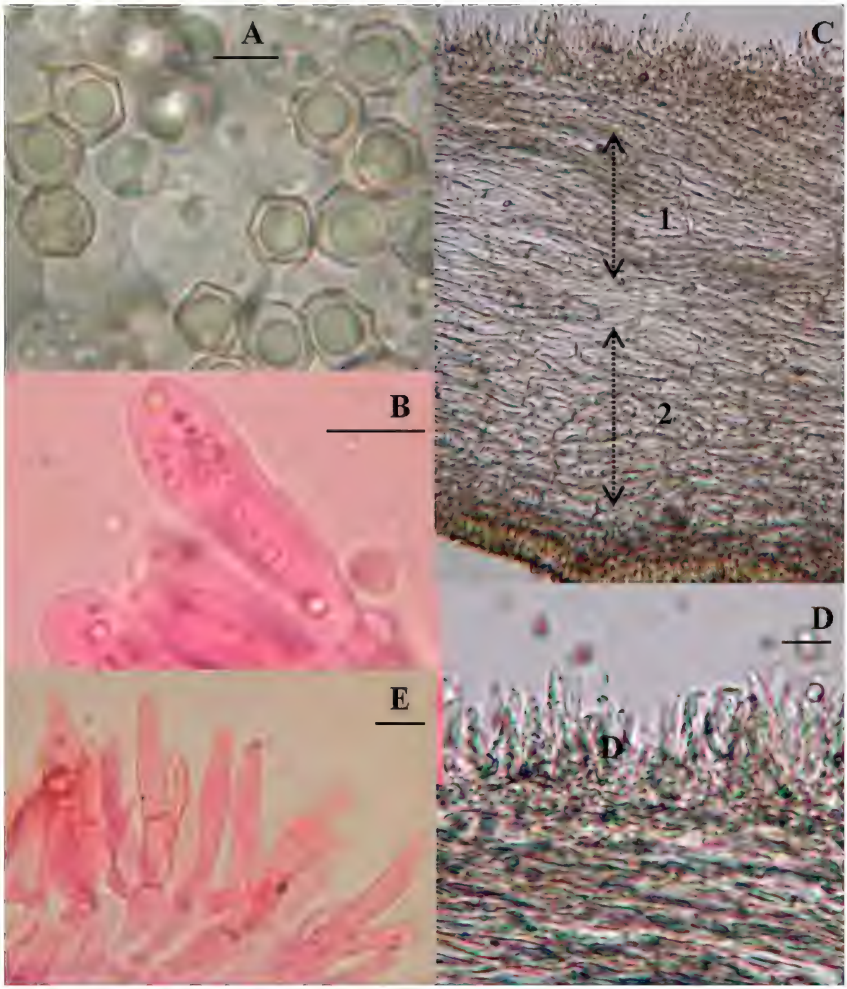


Plate 2. *Entoloma hymenidermum* (DLL10054 holotype). A. Basidiospores. B. Basidium, basidioles. C. Pileipellis radial section at pileus disc; pileal trama cylindric hyphae (top arrows), broad hyphae (bottom arrows). D. Pileipellis (calliderm and outer pileal trama). E. Pileipellis, a calliderm (section slightly squashed). Bars: A = 8 µm; B = 12 µm; C1 = 190 µm; C2 = 230 µm; D = 5 µm; E = 10 µm.

shades of blackish blue, more bluish (20F6) when young, fading slightly (20E4) when drying, and more blackish or dark bluish grey (19E-F3 or 4) when old, background color white, base of the stipe white; context stuffed at first, quickly becoming hollow with maturity; basal tomentum scarce, white. Bruising reactions none.

Basidiospores distinctly 5–6-angular, others are typically collapsed when mounted in 3% KOH, in profile and dorsiventral views isodiametric to subisodiametric, rarely heterodiametric, isodiametric in polar view, 6.8–10.0 (–10.4) × (5.9)6.2–8.8(–9.0) µm (x = 8.3 ± 0.6 × 7.4 ± 0.6 µm; E = 1.00–1.31; Q = 1.12 ± 0.07 (isodiametric); n = 125/4). Basidia narrowly clavate and tapered, relatively long and narrow, 38.9–48.9 × 9.7–14.5 µm (x = 42.8 ± 3.1 × 11.4 ± 1.32 µm; E = 3.25–4.00; Q = 3.78 ± 0.33; n = 16/2); 4-sterigmate. Sclerobasidia absent. Hymenial cystida absent. Lamellar edge fertile. Cheilocystidia and pleurocystidia absent. lamellar tramal hyphae subparallel, in the center to near the subhymenium, mostly relatively long and broad and inflated with rounded end walls, 41.0–170 × 8.5–42.1 µm, rarely long and narrow, not inflated and then 170 × 4 µm. Pileipellis 28.6–89.7 µm in depth, composed of a chain of 3–5 entangled cells, the basal portion developing from the outer layer of the pileal trama and the terminal cells erect, distinct, forming a palisade of pileocystidia that are not tightly packed. Pileocystidia versiform (cylindric, narrowly cylindro-clavate, clavate, obclavate, to ventricose-rostrate with the rostrum up to 2 µm long), 22.4–58.6 × 4.4–18.6 (–21.3) µm (x = 41.6 ± 10.5 × 10.6 ± 4.5 µm; E = 1.20–6.3 (rarely 8.8–9.8); Q = 4.60 ± 2.17; n = 20/2). Pileal trama composed of 2 layers, a layer adjacent to the subhymenium, 228–312 µm in depth composed of inflated, relatively short and broad hyphae, 34.4–137.6 × 9.4–24.2 µm (n = 11/1), and a narrow layer, 119–163 µm deep, composed of cylindric entangled hyphae, 30–110 × 3–6 µm, the outer most portion forming the pileipellis. Stipitipellis a cutis. Caulocystidia and hymenial clusters absent. Oleiferous hyphae present in the subhymenium. Brilliant granules and lipoid globules absent. Pigmentation cytoplasmic in the pileipellis, in 3% KOH at first bluish brown and then colorless due to its solubility in the medium. Clamp connections present in all tissues, small and inconspicuous, easily observed in the branched portion of the pileal tramal hyphae just beneath the pileipellis.

Ecology & distribution — At times abundant, scattered, or solitary in sandy humus with leaf litter, in coastal vine forest near *Maytenus fasciculiflora* Jessup, *Canarium australianum* F. Muell. var. *australianum*, *Polyalthia nitidissima* Benth., and *Guioa acutifolia* Radlk., often adjacent to *Drynaria sparsisora* (Desv.) T. Moore, and at times sheltered by decaying logs and at other times near the mangrove zone, Clifton Beach to Yorkeys Knob Beach, northern Queensland, early to late March.

Additional collections examined — **AUSTRALIA. Queensland**, Cook Region, Clifton Beach, 16°48′50.6″S 145°43′46.6″E, 9 March 2010, PN 016, PN 017; Yorkeys Knob, 16°45′45.7″S 145°40′29.6″E, 29 January 2011, PN 51 3 February 2011, PN 56; 16°48′33″S 145°40′32″E, 8 March 2010, PN 012; 10 March 2010, PN 012A; 16°45′43.8″S 145°40′27.5″E, 14 March 2011, DL Largent 10025.

Distinctive characters — Tricholomatoid basidiomata, minutely velvety bluish black pileus, longitudinally appressed fibrillose dark bluish grey stipe, 2-layered pileal trama, and basidiospores averaging 8 × 7 µm.

Comments — In the phylogeny, *E. hymenidermum* and *E. violaceotinctum* cluster with species that have bluish basidiomata with a finely to distinctly velutinous or tomentose pileus surface, a hymeniform-type (calliderm or erect trichoderm) pileipellis, strongly and distinctly angular basidiospores, and an outer pileus trama of cylindric cells, at least on the disc. Species with a hymeniform type pileipellis are often recognized in *Calliderma* (Aime et al. 2010; Karstedt & Capelari 2010). However, *E. kermandii* G.M. Gates & Noordel., which also falls in this clade, possesses a cutis, a feature inconsistent with the generic circumscription of *Calliderma*. Furthermore, *C. pruinatocutis* (E. Horak) Karstedt & Capelari and *C. rimosum* Karstedt & Capelari (Karstedt et al. 2010) are placed in a distant and unrelated clade (Baroni & Matheny 2011). Without support for the monophyly of *Calliderma*, we classify the species described here as *Entoloma*.

Entoloma hymenidermum is morphologically similar to several species that have a tricholomatoid habit, dark bluish or bluish-black tinged pileus, fibrillose stipe, 5–6 angled basidiospores, clavate to versiform pileocystidia, clamp connections on the pileipellis hyphae, and lack of cheilocystidia.

Entoloma violaceotinctum from New South Wales differs from *E. hymenidermum* in its larger basidiomata with violaceous tones, a stipe that discolors purplish and then brownish when handled, basidiospores averaging 9 × 8 µm, longer (47–73 µm) basidia, and occurrence in deep leaf humus and organically rich soils in a northern warm temperate rain forest. *Entoloma griseolazulinum,* described from Kerala State (India), has a more slender (3–5 mm wide) stipe, longer (10–13 µm) basidiospores, and larger (47–71 × 11–16 µm) basidia (Manimohan et al. 2006). *Calliderma indigoferum* (Ellis) Largent, collected in a swamp among mosses in New Jersey, has an indigo blue (18F3) pileus (75–100 mm broad), white ± blue-tinged blue stipe, and large clamp connections (Largent 1994). *Calliderma caeruleosplendens* Largent et al. (Pakaraima Mountains, Guyana) is distinguished by its densely matted tomentose dark blue pileus, very dark blue stipe, and its broadly clavate, napiform, or broadly obclavate pileocystidia (Aime et al. 2010). *Entoloma rugosopruinatum* Corner & E. Horak, reported from Sabah (Malaysia) and Kerala State (India), differs from all of these species primarily by the clampless hyphae (Horak 1980; Manimohan et al. 1995, 2006).

Entoloma callidermum (Romagn.) Noordel. & Co-David from Africa and Malaysia can be differentiated from *E. hymenidermum* by the violet and/or

bister tinges in the pileus and the stipe, 5–7-angled basidiospores, smaller basidia (27–35 × 6–11 µm), and the absence of a 2-layered lamellar tramal hyphae (Romagnesi 1956, as *Rhodophyllus callidermus*; Morgado et al. 2013). However, the original description provided by Romagnesi (1956) may include segregate species (Eyssartier et al. 2012).

Calliderma fibulatum Karstedt & Capelari, *Entoloma simillimum* Corner & E. Horak, *E. marinum* Corner & E. Horak, *E. ducale* E. Horak, *E. divum* Corner & E. Horak, and *E. burkilliae* Massee resemble *E. hymenidermum* and *E. violaceotinctum* in basidiome color and the pileipellis but differ in their 5–11 µm broad fusoid to cylindric pileocystidia and the presence of cheilocystidia (Horak 1980, Karstedt & Capelari 2010). *Entoloma coeruleomagnum* G.M. Gates & Noordel. differs by its palisadoderm pileipellis and *E. kermandii* by its cutis (Noordeloos & Gates 2012).

Entoloma violaceotinctum Largent, **sp. nov.** PLATES 3-4

MYCOBANK MB804380

Differs from *Entoloma hymenidermum* by its pileus and stipe with violet or violaceous tones, stipe that bruises when handled, larger basidiospores, and longer basidia.

TYPE — Australia, New South Wales, Strickland State Forest, lower track, central Hunter District, within 20m of 33°22′49″S 151°19′32″E, 14 April 2011, DL Largent 10088 (**holotype** DAR).

ETYMOLOGY — Derived from the Latin *violaceus* (= violet) + *tinctus* (= tint) for the violet tinted pileus and stipe.

PILEUS 40–100 mm broad, 7–40 mm high; opaque and even, hygrophanous, not translucent-striate; broadly umbonate at all times, convex to broadly convex at first, then plane to uplifted with age; velvety, at times appearing stippled with minute, pointy hairs; dark violet to blackish blue (18F4–5 or 19F5–6) fading to dull violet to dark blue (18–19E4–5) with a darker center (dark violet grey 17F2), dull; margin incurved then decurved, entire; context cream-colored except for the pellicle that is concolorous with the surface, 6–10 mm broad above the stipe, 1–3 mm broad at the margin. ODOR mild and fragrant in young specimens, strong but undefined in older specimens. TASTE indistinct in younger specimens, unpleasant in older specimens. LAMELLAE 15–45 mm long, 6–15 mm deep, moderately broad; adnexed and close then sinuate and subdistant, seceding with age; at first white or off-white to pale orange (5A3 or 15A2–3) then light orange (5A4) with basidiospore maturation; margin smooth and concolorous. STIPE 40–92 mm long, 6–16 mm broad at apex, 5–13 mm broad at base, at times flattened and then 17 × 7 mm at the apex and 13 × 10 mm at the very base, versiform in shape (equal, subclavate, clavate or broad at apex and more narrow at base); appressed fibrillose; fibrils medium

Plate 3. *Entoloma violaceotinctum* (DLL10088 holotype). A. Basidiomata. B. Stipe surface. C. Pileus surface. Bar: A = 20 mm; Bar scale: B. = 16 mm; C = 35 mm.

violet grey (18D2–3), base color yellowish white to light orange (4A2 or 5B3); context white, at first stuffed with maturity becoming hollow and then snaps easily; basal tomentum absent. Bruising reactions on the stipe occurs after handling, becoming purplish (18E4) and then brownish (5E4).

Basidiospores 5–6-angled, angles typically distinct, often with irregularly wavy walls in dried specimens, isodiametric to subisodiametric in profile and dorsiventral views, isodiametric in polar view, 7.8–10.2 × 7.1–9.2 µm (x = 8.9

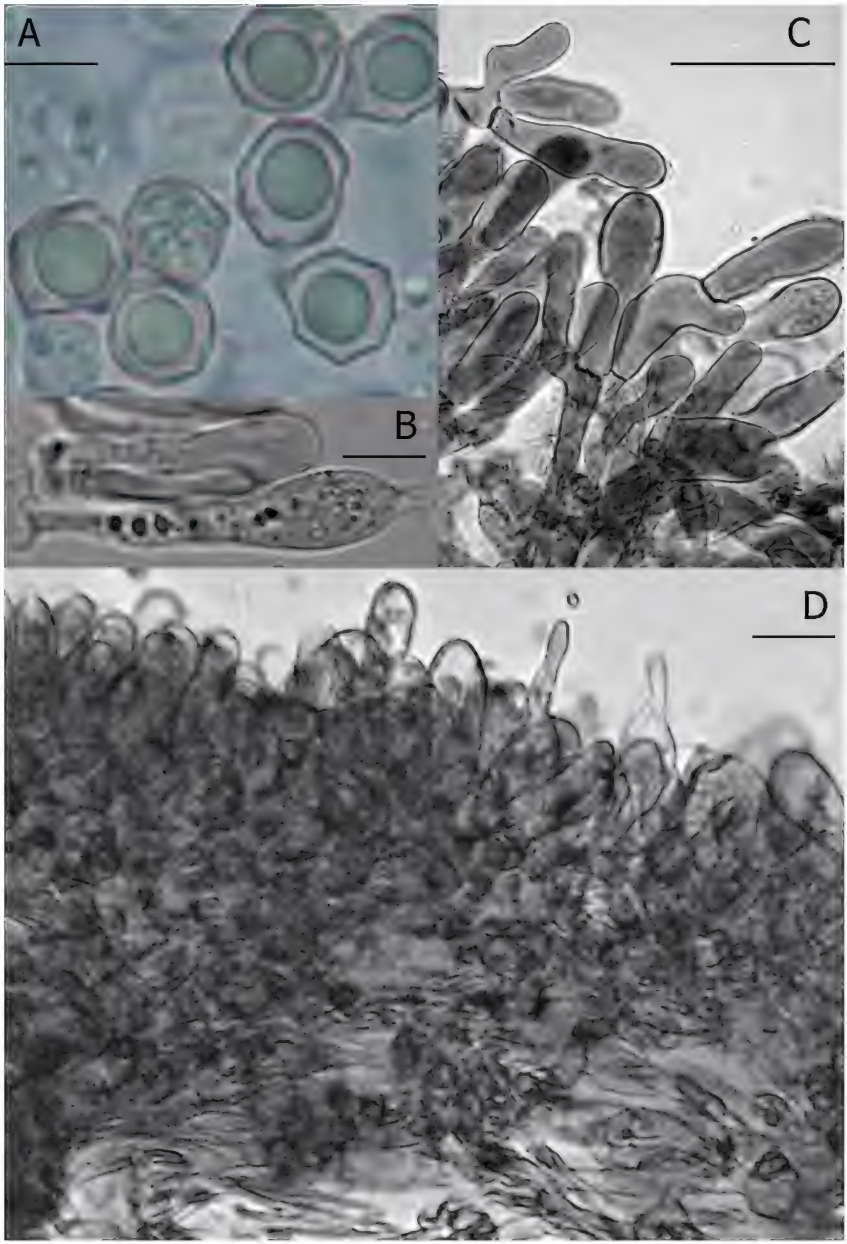


Plate 4. *Entoloma violaceotinctum* (DLL10088 holotype). A. Basidiospores. B. Basidia, basidioles. C. Pileipellis branching pattern. D. Pileipellis (calliderm and outer pileal trama). Bars: A = 8 µm; B = 13 µm; C, D = 30 µm.

± 0.7 × 8.0 ± 0.7 µm; E = 1.00–1.24; Q = 1.11 ± 0.06 (isodiametric); n = 56/2). Basidia clavate, very long and relatively narrow, often tapered to 2–3 µm, usually with rather large droplets or globules in mature forms, 4-sterigmate, 47.1–73.1 × 10.8–14.2 µm (x = 57.4 ± 7.8 × 12.6 ± 0.9 µm; E = 3.63–5.77; Q = 4.57 ± 0.58; n = 25/2). Sclerobasidia absent. Hymenial cystidia absent. Lamellae in section composed of two layers, a hymenium 31–45 µm thick, subhymenium 13–22 µm thick with two lateral strata, each 17–30 µm thick, and a central layer (86–95 µm wide). Lamellar trama hyphae subparallel, relatively short, consisting of two types, slender, narrow hyphae, 61.2–113.1 × 3.3–8.0 µm (n = 9/2) forming between the subhymenium and the center of the lamellae and inflated hyphae, 39.5–105.3 × 9.5–22.2 µm (n = 22/2) found in the center. Pileipellis 36–54 µm in depth, a hymeniderm of pileocystidia, composed of entangled layer of hyphae, typically 3 cells long, with the pileocystidia as 1–3 terminal cells branching from a subterminal cell. Pileocystidia the terminal cells of branched hyphae that originate from the outer pileal trama, clavate to broadly obclavate, 29.8–82.7 × 8.2–39.3 µm (x = 48.9 ± 12.3 × 14.9 ± 4.5 µm; E = 1.01–6.36; Q = 3.55 ± 1.14; n = 38/2). Pileal trama composed of two layers, an entangled layer (61–210 µm thick) with slender hyphae, 68.6–112.6 × 3.7–9.1 µm; (n = 7/1) the outer portion of which produce the pileipellis and an adjacent layer (475–539 µm thick) composed mostly of inflated hyphae, 50–237 × 12.5–26.0 µm (n = 15/2). Stipitipellis a cutis. Caulocystidia and hymenial clusters absent. Oleiferous hyphae present in the trama of the lamellae and the pileus. Brilliant hyphae and lipoid globules absent. Pigmentation cytoplasmic in the pileipellis, in 3% KOH at first bluish brown and then colorless due to its solubility in the medium. Clamp connections rather large and present in all tissues.

Ecology & distribution — Typically gregarious in mid-April, then scattered in early May, in deep leaf humus and organic rich soil, near *Syncarpia glomulifera* (Sm.) Nied., *Ceratopetalum apetalum* D. Don, and *Doryphora sassafras* Endl.), and *Schizomeria ovata* D. Don hosts, New South Wales, central Hunter District, Strickland State Forest, mid April through early May.

Additional collections examined — **AUSTRALIA. New South Wales**, central Hunter District, Strickland State Forest, lower car park area, 19 April 2011, DL Largent 10128; 33°22′47″S 151°19′28″E, DL Largent 10129; 33°22′47″S 151°19′27″E, 28 April 2011, DL Largent 10168; 33°22′47″S 151°19′28″E; 8 May 2011, DL Largent 10215; 33°22′48″S 151°19′30″E, *Banksia* loop path, 19 April 2011; 33°22′19″S, 151°19′11″E, 3 May 2011, DL Largent 10183.

Distinctive characters — Tricholomatoid basidiomata with violaceous tones, a stipe that discolors purplish then brownish when handled, basidiospores averaging 9 × 8 µm, long (47–73 µm) basidia, and its occurrence in deep leaf humus.

Comments — Refer to the Comments section under *E. hymenidermum* for a discussion of the differences among morphologically similar species.

Entoloma discoloratum Largent, **sp. nov.** Plate 5

MycoBank MB804381

Differs from *Entoloma albomagnum* by its orangish pileus disc, appressed fibrillose stipe discoloring yellowish then brownish, broader lamellae, farinaceous odor without a soapy component, and sclerobasidia.

Type — Australia, New South Wales, Central Hunter District, Barrington Tops National Park, within 20 m of 32°14′25.2″S 151°43′29.8″E, 25 April 2010, DL Largent 9936 (**holotype** DAR).

Etymology — Derived from the Latin *discoloratus* (= discolored) for the discoloration of the pileus and stipe.

Pileus 64–80 mm broad, 11–18 mm high; not hygrophanous, not translucent-striate; broadly convex and broadly umbonate becoming irregularly uplifted with age; mostly glabrous but obscurely fibrillose in places particularly near the margin, dull, sticky to the touch; orange white to pale orange to light orange (5A3–4or 5A2–3) in the center, off-white elsewhere; margin decurved at first becoming plane and then uplifted, entire and rimose then quickly eroded; context white, 3–8 mm deep above the stipe, nearly non-existent at the margin. Odor of mature basidiomata distinctly farinaceous, particularly after being crushed. Taste strongly farinaceous. Lamellae 20–35 mm long, 7–15 mm deep; moderately broad to broad at first and when expanded and mature, mostly sinuate, in a few places broadly adnexed or adnate; at first crowded or close and then nearly subdistant; off-white (5A2–3); margin smooth and concolorous. Stipe 67–148 mm long, 10–21 mm broad at apex, 10–14 mm broad in the middle, 13–15 mm broad at base, tapered at the base or ventricose in the middle and then tapered; decidedly and deeply appressed fibrillose along the entire surface; off white, solid and stuffed; basal tomentum absent. Bruising reactions pileus stains or bruises greyish orange (5B4); stipe discoloring yellowish then brownish (5A2 to 5B3 or 5E-F4) when handled.

Basidiospores distinctly angular, isodiametric to subisodiametric in profile view, 6.7–8.3 × 5.8–8.5 µm (x = 7.5 ± 0.48 × 7.0 ± 0.59 µm; E = 0.92–1.20; Q = 1.07 ± 0.08 (isodiametric); n = 31/1). Basidia cylindro-clavate, slender, 42.1–50.3 × 7.6–10.3 µm (x = 46.0 ± 3.04 × 8.9 ± 0.89 µm; E = 4.43–6.09; Q = 5.2 ± 0.61; n = 7/1), full of large irregularly shaped globules; 2– or 4-sterigmate. Sclerobasidia rare, aborted 51–53.9 × 8.4–10.7 µm, wall 1.7–2.3 µm thick (n = 2/1), filled with large globules. Hymenial cystidia absent. Lamellar tramal hyphae subparallel, relatively long and broad, 63.2–199.7 × 13.0–29.9 µm (n = 7/1). Pileipellis 140 µm thick, an entangled ixocutis, particularly evident near the center of the pileus, hyphae just below the surface, 3.3–5.8 µm wide (n = 4/1). Pileocystidia mostly prostrate, a few semi-erect, slender,

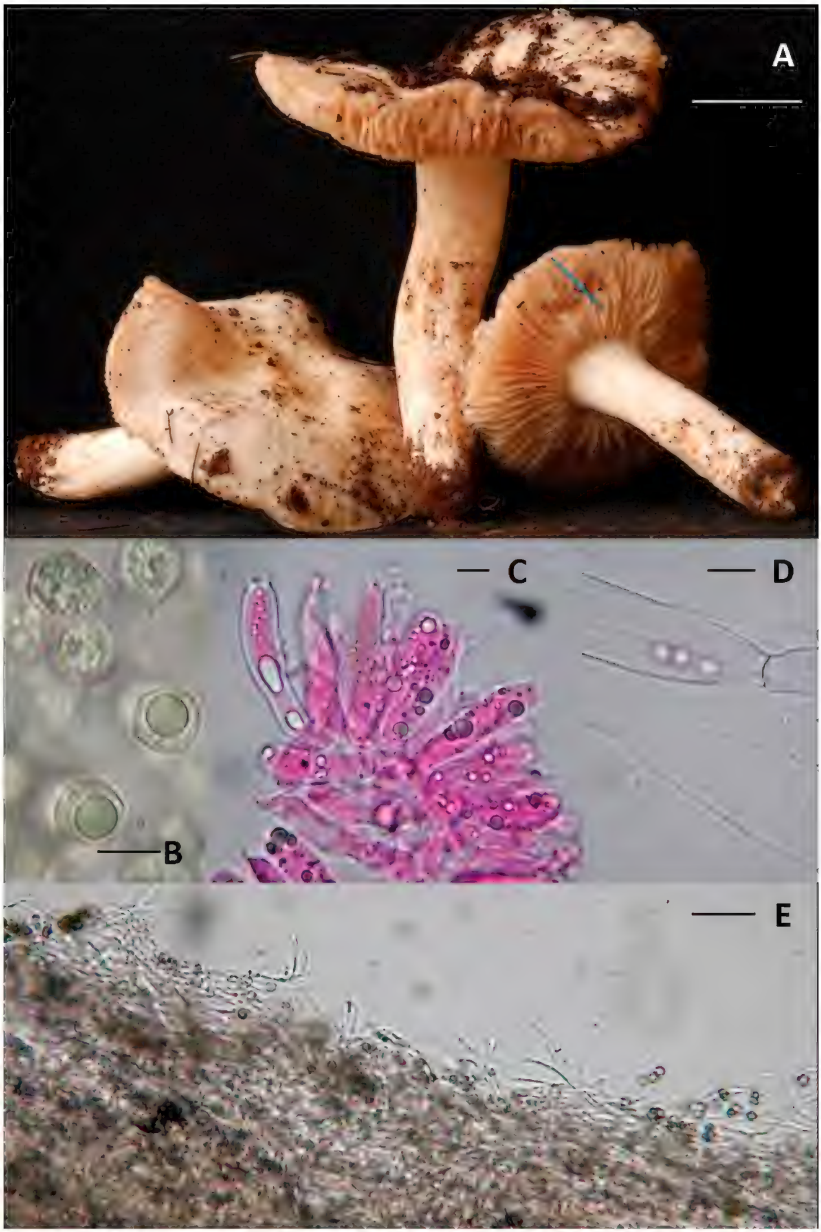


Plate 5. *Entoloma discoloratum* (DLL 9936 holotype). A. Basidiomata. B. Basidiospores. C. Sclerobasidium on left; elsewhere thin-walled basidia and basidioles (1% phloxine stain). D. Hyphae of lamellar trama, large clamp on septum of top hyphae. E. Pileipellis, an ixocutis. Bars: A = 20 mm; B = 7 µm; C = 10 µm; D = 15 µm; E = 35µm.

cylindric, 40.9–45.0 × 2.0–3.0 µm (n = 4/1). Pileal tramal hyphae similar to the lamellar trama, 76.2–169.3 × 16.9–35.3 µm (n = 7/1). Stipitipellis a cutis. Caulocystidia and hymenial clusters absent. Oleiferous hyphae abundant in the lamellar trama, end cells may extend into the subhymenium. Brilliant granules and lipoid bodies absent. Pigmentation non-existent. Clamp connections abundant at the base of the basidia, on the hyphae of the lamellar trama and pileipellis.

Ecology and distribution - Scattered to gregarious and buried in the soil and leaf litter, forming a fairy ring around *Syncarpia glomulifera* and near *Allocasuarina torulosa* (Aiton) L.A.S. Johnson, New South Wales, Barrington Tops National Park, Jerusalem Creek Track, late April to early-mid May.

Additional collections examined — **AUSTRALIA. New South Wales**, central Hunter District, Barrington Tops National Park, Jerusalem Creek Track. 32°14′25.2″S 151°43′29.8″E, 10 May 2010, DL Largent 9970 (**topotype** DAR); 32°14′23″S 151°43′29″E, 10 May 2011, DL Largent 10217.

Distinctive characters — Large off-white, subviscid pileus with pale yellow orange colors in the center; strongly fibrillose stipe without fibrillose flocks that eventually bruises yellowish then brown, sclerobasidia, distinctly angular basidiospores, and an ixocutis-type pileipellis.

Comments — *Entoloma discoloratum,* which clusters with *E. albomagnum* and *E. cretaceum,* can be classified in *Entoloma* sect. *Albida* Noordel. based on its white basidiomata (Noordeloos & Gates 2012).

Entoloma albomagnum is distinguished by its white pileus with faint yellow tinge at the center, silky fibrillose-scurfy white stipe that does not discolor, soapy odor when first collected, weakly angular basidiospores, lamellar and pileal trama composed of short barrel-shaped elements (20–90 × 4–15 µm), and the absence of sclerobasidia (Noordeloos & Gates 2012). The similarly white *E. cretaceum* from Tasmania differs in its smaller chalky white basidiomata, the white watery fibrous stipe, and rancid-farinaceous odor (Gates & Noordeloos 2007, Noordeloos & Gates 2012).

Entoloma kewarra Largent, **sp. nov.** Plate 6

MycoBank MB804382

Differs from *Entoloma praeluteum* by its subviscid pileus with a pruinose bloom at first, fibrillose stipe base, white context becoming greenish yellow, ixocutis, and the intracellular pigment in the pileipellis.

Type — Australia, Queensland, Cook region, Kewarra Beach coastal vine forest. Within 20 m of 16°46′50.7″S 145°41′24.8″E, 12 March 2012, DL Largent 10275 (**holotype** BRI, **isotype** CNS).

Etymology — referring to the type locality, Kewarra Beach coastal vine forest.

Pileus 6–52 mm broad, 4–12 mm high; opaque, not hygrophanous, not translucent-striate; when immature conic-campanulate then convex, finally broadly convex to plane, at times broadly umbonate; at first entirely covered with a thin layer of yellowish white fibrils (2–3A-C7–8), with maturity the bloom showing spots when damaged or dry, eventually the surface becomes glabrous but sticky to the touch when bloom disappears; surface without bloom yellow (4A-B7) except light yellow (3 or 4A2–4) on the margin, when old and overly mature becoming entirely olive yellow (3C8); margin entire, at first incurved, when mature remaining incurved or becoming decurved; context yellowish white (3A3) becoming vivid yellow (2–3A7–8) above the stipe and slightly lighter (3A7) elsewhere, solid, and 2–6 mm thick above the stipe, 1 mm thick at the margin. Odor farinaceous and somewhat pungent. Taste bitter and farinaceous. Lamellae 2–17 mm long, 1–5 mm deep; in some narrowly adnate at first then adnexed, in most adnexed at all times, crowded then close, narrow at first then slightly more broad in some; when immature yellowish white to light yellow (4A2–3) becoming orange yellow (5A2) then light orange (4–5A4–5) with basidiospore production; margin smooth and concolorous. Stipe 25–100 mm long, 4–10 mm broad at the apex, in some flattened and 5 × 8, 10 × 13, or 13 × 15 mm broad at the apex, 5–15 mm broad in the middle, and 4–13 mm broad at the base, tapered; distinctly appressed-fibrillose; pale yellow to light yellow (2A2–3, 3A3, 4A4) becoming olive yellow (3C8) with age; context solid and stuffed then becoming hollow from the apex downward, white at first becoming greenish yellow on exposure and when cut becoming yellow (3A6–7) where hollow; basal tomentum absent. Bruising reactions stipe surface becoming pale yellow to light yellow (4A3–4) then olive or olive brown (3E-F2) where handled.

Basidiospores distinctly angular in larger basidiospores, indistinctly angular in smaller basidiospores, isodiametric to barely subisodiametric in all views, short hilar appendage, 5.3–6.8 × 5.0–6.4 µm (x = 6.4 ± 0.3 × 6.0 ± 0.3 µm; E = 1.00–1.19; Q = 1.07 ± 0.05 (isodiametric); n = 32/1). Basidia with abundant granules, narrowly clavate and hardly tapered, 25.7–37.5 × 7.2–11.8 µm (x = 31.5 ± 4.9 × 8.9 ± 1.2 µm; E = 2.74–4.94; Q = 3.61 ± 0.71; n = 14/2); 4-sterigmate, the sterigma short and up to 2 µm long. Sclerobasidia absent. Hymenial cystidia absent. Lamellar tramal hyphae subparallel, slightly narrow, 63.3–101.2 × 5.4–9.7 µm (x = 76.0 ± 11.6 × 7.5 ± 1.62 µm; E = 7.28–15.86; Q = 10.6 ± 2.78; n = 10/1). Pileipellis an entangled ixocutis, 29.0–120.0 µm in depth. Pileocystidia narrowly cylindro-clavate, at times with a slightly inflated and minutely rostrate apex, 13.9–29.5 × 1.6–2.9 µm (n/1 = 6). Pileal tramal hyphae relatively short and narrow, 33.9–106.9 × 3.7–14.9 µm (x = 55.8 ± 24.0 × 9.4 ± 3.2 µm; E = 3.16–12.65; Q = 6.67 ± 3.37; n = 12/1). Oleiferous hyphae and lipoid globules absent. Pigmentation

Plate 6. *Entoloma kewarra* (A-B DLL 10275 holotype; C-E DLL 10055). A. Basidiomata. B. Pileus surface with bloom of very finely appressed fibrils on pileal margin. C. Basidiospores. D. Basidium and basidioles (1% phloxine stain). E. Pileipellis, an ixocutis. Bar scale: A, B = 15; mm; Bars: C = 6 µm; D = 9 µm; E = 30 µm.

intracellular and yellowish in the pileipellis, exudes a yellowish green exudate in 70% ethanol, water and 3% KOH. Clamp connections present on all the tissues, narrow and small.

Ecology and distribution —Solitary to scattered in the open and in sand between a poorly defined heath community and coastal vine forest near a mangrove community in northern Queensland, Kewarra Beach, late January through late March.

Additional collections examined — **AUSTRALIA. Queensland,** Cook Region, Kewarra Beach, 16°46′49.7″S 145°41′23.1″E, 28 January 2011, PN 50; 4 February 2011, PN 57; 16°49′00.3″S 145°43′49.3″E, 21 March 2011, DL Largent 10055; 16°46′50.8″S 145°41′24.6″E 18 March 2012, DL Largent 10289, PN 70; DL Largent 10290, PN 72.

Distinctive characters — Basidiomata tricholomatoid and buried in the sandy substrate up to two-thirds the stipe length, pileus initially with a yellowish white pruinose bloom, subviscid pileus surface, stipe surface light or pale yellow becoming darker where handled, stipe context becoming greenish yellow with exposure, an ixocutis-type pileipellis, and narrowly clavate basidia.

Comments — *Entoloma pamelae* and *E. kewarra* are strongly supported as sister taxa in our analyses. With its bright yellow colors and tricholomatoid basidiomata, *E. pamelae* (also from northeastern Queensland) differs in its shiny dry egg-shell smooth glabrous pileus, diffracted-scaly stipe base, white context turning yellow, narrower basidia, minutely externally encrusted pigmentation in the pileipellis, and cutis-type pileipellis. Additional *Entoloma* species with similar morphological features are discussed in the Comments section under *E. pamelae*.

Entoloma pamelae Largent, **sp. nov.** Plate 7

MycoBank MB804383

Differs from *Entoloma praeluteum* by its smaller pileus and stipe, fibrillose-squamulose stipe base, longer basidia, mild and indistinct odor and taste, and narrow cheilocystidia.

Type — Australia, Queensland, Cook region, Danbulla National Park, Kauri Creek Track, within 20 m of 17°07′50.6″S 145°35′57.7″E, 28 February 2010, DL Largent 9753 (**holotype** BRI, **isotype** CNS).

Etymology — in honor of Pamela Largent, the collector of the holotype.

Pileus 11–40 mm broad, 5–15 mm high; opaque, not translucent-striate, hygrophanous; convex when young and not umbonate, upon expansion and maturity becoming broadly umbonate to faintly gibbous and either remaining convex or becoming broadly convex; shiny, moist, at all times decidedly glabrous and smooth like an egg-shell; at first yellow (3A6 or 7) when hygrophanous darker yellow (3B6 or 6–7, "mustard yellow" or "Nankeen yellow"); margin entire becoming broadly lobed, decurved; context (beneath a thick yellow rind) 5–7 mm deep above the stipe, <1 mm at the very margin,

Plate 7. *Entoloma pamelae* (DLL9753 holotype) A. Basidiomata. B. Basidiospores. C. Basidia (1% phloxine stain). D. Cheilocystidia (1% phloxine stain). E. Pileipellis, a cutis. F. Pileocystidium with faint external incrustations (arrow). Bars: A = 20 mm; B, C = 5 µm; D = 20 µm; E = 15 µm; F = 3 µm.

yellow white (3A2) becoming brighter yellow (3A2–3) then pale yellow (3A3) upon exposure to air. ODOR and TASTE indistinct and mild. LAMELLAE 2–20 mm long, 0.5–4.0 mm deep, when young consistently narrow then moderately broad to sigmoid; at first adnexed with maturity remaining adnexed but at times becoming sinuate, crowded then close; yellowish at first becoming pinkish with basidiospore maturity and then distinctly contrasted when compared with the pileus and stipe colors; margin concolorous with the face, smooth, at times faintly denticulate. STIPE 32–60 mm long, 5–15 mm broad at apex, 4–8 mm broad at base; consistently narrowing to the base; at first faintly appressed-fibrillose becoming distinctly-appressed fibrillose with the fibrils matted and becoming ± fibrillose-squamulose towards base; pale yellow to pastel yellow then dull yellow (3 or 4A3–4), the darker colors more evident on handling; context at first hollow in the apical ⅓ to ¼ and stuffed elsewhere, upon maturity becoming mostly hollow, white when first cut, quickly becoming yellow upon exposure to air; basal tomentum absent. BRUISING REACTIONS stipe bruises brownish on handling.

BASIDIOSPORES 5-angled in profile view, 4–5-angled in dorsiventral view, distinctly angular, small, isodiametric to heterodiametric, 5.0–7.6 × 5.1–7.3 µm (x = 6.4 ± 0.53 × 6.0 ± 0.49 µm E = 0.83–1.35; Q = 1.07 ± 0.11 [isodiametric]; n = 30/1). BASIDIA cylindric to cylindro-clavate and very narrow 33.7–38.7 × 5.1–7.7 µm (x = 35.6 ± 1.8 × 6.6 ± 0.8 µm; E = 4.60–6.56; Q = 5.4 ± 0.55; n = 9/1); 4–sterigmate. SCLEROBASIDIA absent. HYMENIAL CYSTIDIA absent. CHEILOCYSTIDIA cylindric to narrowly obclavate, 24–37.7 × 0.8–4.1 µm (n = 6/1), the ends of lamellar tramal hyphae protrude through the hymenium. LAMELLAR TRAMAL HYPHAE subparallel, very narrow, 55.1–113.1 × 2.7–12.9 µm (n = 6/1). PILEIPELLIS a periclinally arranged cutis composed of narrow, cylindric hyphae, 10–30 µm deep and with an unknown material on the outer surface that stains golden yellow in 3% KOH; PILEOCYSTIDIA cylindric to narrowly cylindro-clavate, 24–40 × 2.2–3.6 µm. PILEAL TRAMAL HYPHAE not studied. STIPITIPELLIS a cutis. CAULOCYSTIDIA and HYMENIAL CLUSTERS absent. OLEIFEROUS HYPHAE, BRILLIANT GRANULES, and LIPOID GLOBULES absent. PIGMENTATION minutely incrusting the outer walls of the pileipellis hyphae. CLAMP CONNECTIONS present at the base of the basidia, the cheilocystidia, and on the hyphae of the lamellar trama and pileipellis.

ECOLOGY AND DISTRIBUTION — Scattered to gregarious in leaf humus beneath an unidentified species of *Casuarina* or *Acacia* in a dry sclerophyll forest in northern Queensland, Danbulla National Park, Kauri Creek Track from mid-February to late March.

ADDITIONAL COLLECTIONS EXAMINED—**AUSTRALIA. QUEENSLAND**, Cook Region, Danbulla National Park, Kauri Creek Track, 17°07′59.1″S 145°35′55.6″E, 2 March

2010, DL Largent 9763; 17°07′56.3″S 145°35′54.3″E 17 March 2010, DL Largent 9787; 17°08′02.4″S 145°35′51.7″E, 27 March 2012, DL Largent 10352.

Distinctive characters — Basidiomata yellow in contrast to the pinkish mature lamella, narrow yellowish immature lamellae, pileus glabrous and smooth like the surface of an egg, pileal context pale yellow beneath a thick bright yellow rind, small (x = 6.4 × 6.0 µm) distinctly angular basidiospores, cylindric to obclavate cheilocystidia, minutely encrusted pigment on the external pileipellis hyphal walls.

Comments — *Entoloma praeluteum* Corner & E. Horak (from Malaysia) differs from *E. pamelae* by the larger (40–80 mm broad) pileus, longer (50–80 mm) stipe, farinaceous odor, shorter (25–30µm) basidia, and lack of cheilocystidia (Horak 1980).

Entoloma flavidum (Massee) Corner & E. Horak (from Singapore, Malaysia, and Kerala, India) and *E. xanthomyces* Corner & E. Horak (from Singapore) have somewhat similar basidiome colors and stature as *E. pamelae* but differ in their broader (≥140 mm) pileus and white to pallid immature lamellae that become subdecurrent or decurrent with age. Additionally, in *E. flavidum* the basidiospores are larger (9–11 × 6.5–7.5 µm) and the basidia are longer (25–65 mm long) than those of *E. pamelae* (Horak 1980; Manimohan et al. 1995, 2006).

Entoloma rugosiviscosum Largent **sp. nov** Plates 8–9

MycoBank MB804384

Differs from *Entoloma conspicuum* by its smaller basidiomata, the radial rugose viscid pileus, brown stipe, and ixotrichodermium.

Type — Australia, Queensland, Cook region, Daintree National Park, Tribulation section, Marrjda Track, within 20 m of 16°08′18.5″S 145°26′26.0″E, 20 March 2009, DL Largent 9676 (**holotype** BRI, **isotype** CNS).

Etymology — from the Latin adjectives *rugosus* + *viscosus* referring to rugose viscid pileus surface.

Pileus 55–75 mm broad, 15 mm high; not hygrophanous, not striate, and not translucent; broadly convex and umbonate; shiny, viscid and sticky to the touch and remaining so 14 hours after collecting; scummy on the disc, radially-rugose elsewhere; medium dark yellow brown (5E-F5) on the disc and brownish orange to light brown (5C-D4–5) elsewhere, eventually entirely greyish orange with age and fading; margin greyish orange (5B2–3), decurved and even, elastic, and extends past lamellae up to 2 mm; context solid, brownish beneath the cuticle, white elsewhere, 10 mm broad above the stipe apex, 1–1.5 mm at the margin. odor mild to mildly pungent. Taste mild. Lamellae 25–26 mm long, 11–13 mm deep; uncinate to adnexed, at times nearly free; white at first; subdistant, more or less ventricose and broad; margin smooth then eroded with age,

Plate 8. *Entoloma rugosiviscosum* (DLL9676 holotype). A. Basidiome with rugose pileus. B. Lamellae with eroded margins and stipe apex. Bar = 10 mm.

concolorous. Stipe 50–51 mm long, 11–14 mm broad at the apex, 9.5–14 mm broad in the middle, 7–15 mm broad at the base, more or less clavate-tapered, white at first discoloring yellowish (3A2–3) with age and handling, minutely

Plate 9. *Entoloma rugosiviscosum* (A. DLL9676 holotype; B–D. DLL 9644, 1% phloxine stain). A. Basidiospores. B. Basidia, basidioles, clamp connection. C. Lamellar trama with gelatinized subhymenium, section slightly squashed. D. Pileipellis, an ixotrichodermium. Bars: A = 5 µm; B = 8 µm; C, D = 40 µm.

appressed-fibrillose; flesh white, fibrous-stuffed then hollow in the center with age. Bruising reactions basidiomata discoloring yellowish with handling.

Basidiospores typically cuboidal and distinctly angular in all views, rarely 5–sided, small, 4.8–7.0 × 4.1–6.6 µm (x = 5.8 ± 0.4 × 5.4 ± 0.5 µm; Q 0.87–1.25; E 1.07 ± 0.08; n = 52/2). Basidia 4-sterigmate, long and narrow, 33.1–44.7 × 6.4–9.7 µm (x = 38.1 ± 3.4 × 7.99 ± 1.1 µm; Q 3.76–6.23; E 4.85 ± 0.8; n=10/1. Sclerobasidia absent. Hymenial cystidia absent. Lamellar trama 318–400 µm wide; subhymenium gelatinized and broad, 40–56 µm wide. Lamellar tramal hyphae subparallel to nearly parallel, short and broad, 38.5–102.2 × 12.7–17.6 µm. Pileipellis an ixotrichodermium with cylindric hyphae 2–3

μm diam., walls irregular and gelatinized. Pileal tramal hyphae subparallel, relatively short and broad, 61.2–133.5 × 15.2–31.0 μm. Stipitipellis a cutis but with solitary but scattered basidia-like cells at the apex. Stipe tramal hyphae nearly parallel, narrow in the outer portions, short and broad in central area and then 81.7–169.8 × 15.2–36.9 μm. Oleiferous hyphae abundant in the trama of the pileus, lamellae, and stipe. Brilliant granules and lipoid globules absent. Pigmentation cytoplasmic, brownish and uniform in the hyphae of the pileipellis. Clamp connections abundant and present in all tissues.

Ecology & distribution — Solitary, humicolous, adjacent to the pavement on the Marrdja Track, 15–50 m from parking lot, Daintree National Park, Tribulation Section.

Additional collections examined — **AUSTRALIA. Queensland**, Cook Region, Daintree National Park, Tribulation section, Marrjda Track, 16°08′18.5″S 145°26′26.0″E, 12 March 2009, DL Largent 9644.

Distinctive characters — Radially rugose viscid yellow brown pileus that fades to brownish orange or light brown, white stipe discoloring yellowish, basidiospores mostly cuboidal and small (4.8–7.0 × 4.1–6.6 μm), an ixotrichodermium-type pileipellis, long narrow basidia.

Comments — Phylogenetically *E. rugosiviscosum* clusters with several species including *E. prunuloides* (Fr.) Quél., *E. albomagnum,* and *E. cretaceum.* Excluding *E. ochreoprunuloides* Morgado & Noordel.), most of these species possess a tacky, sticky, subviscid, viscid, or glutinous pileus surface with an ixocutis- or ixotrichodermium-type pileipellis. *Entoloma conspicuum* E. Horak from Papua New Guinea resembles *E. rugosiviscosum* in its tricholomatoid basidiomata, brown rugose to subvenous pileus, and cuboidal basidiospores but differs by the cutis-type pileipellis and larger basidiomata (Horak 1976, 1980). *Entoloma rugosostriatum* Largent & T. W. Henkel from Guyana also has a brown rugose pileus and tricholomatoid stature but differs in its isodiametric nodulose basidiospores (Largent et al. 2008).

Entoloma guttulatum Largent, **sp. nov.** Plates 10–11

MycoBank MB 807410

Differs from *Entoloma pingue* by the brown pileus, lamellae with discolored spots, larger basidia, and unusual pileipellis.

Type — Australia, Queensland, Cook region, Mossman National Park, 20 m from 16°28′17.1″S 145°19′51.2″E, 18 March 2010, DL Largent 9791 (**holotype** BRI, **isotype** CNS).

Etymology — from the Latin adjective *guttulatus* referring to droplets on the lamellar edge that dry as reddish brown spots

Pileus 80–82 mm broad, 20 mm high; opaque, not hygrophanous, not translucent-striate; not umbonate, convex then uplifted; moist, glabrous to

Plate 10. *Entoloma guttulatum* (DLL9791 holotype). A. Basidiome. B. Pileus surface. Bar = 13 mm.

the eye but under 4× to 20× subfelty or suede-like and with minute colorless pruinae; between greyish brown and yellowish brown (5D-E3–4) fading to between light orange and grayish orange (5A-B5–6) when old; margin plane then uplifted, wavy and crenulate. Odor and Taste unpleasant and slightly farinaceous. Lamellae 30–35 mm long, 9–10 mm deep, broad; adnate but a decurrent or subdecurrent tooth or subdecurrent; greyish orange (6B4) when mature; margin smooth and concolorous, with droplets as in some *Hebeloma* sp. that are abundant and dry as reddish brown spots. Stipe 60–100 mm long, 20 mm broad at the apex, 14 mm broad at base; longitudinally appressed fibrillose; grayish orange (6B4); context stuffed becoming hollow with age; basal tomentum absent. Bruising reactions stipe surface developing brownish orange to light brown (6C-D4) areas with age and handling.

Basidiospores distinctly angular, 5–6-angled, isodiametric to subisodiametric in profile view, isodiametric in polar view, 7.0–9.2 × 6.7–8.8 µm (x = 8.1 ± 0.6 × 7.8 ± 0.5 µm; E = 0.9–1.3, Q = 1.04 ± 0.08 (isodiametric); n = 60/2). Basidia long and cylindro-clavate, 45.7–60.9 × 7.4–11.2 µm (x = 53.5 ± 5.7 × 9.6 ± 1.3 µm; E = 4.79–6.16, Q = 5.64 ± 0.56; n = 6/1); 2- or 4-sterigmate. Sclerobasidia absent. Hymenial cystidia absent. lamellar tramal hyphae 57.6–112.2 × 8.8–13.6 µm (n = 7/1). Pileipellis 48–70 µm deep, a colorless anticlinal layer of slender, entangled hyphae, 3-4 µm in diam, beneath which is a pigmented, densely entangled layer of hyphae; terminal cells cylindric to narrowly obclavate to rostrate-ventricose, 28.2–43.0 × 3.2–5.3 µm (n = 6/1). Pileal tramal hyphae composed of broad cells. 34.9–59.9 × 7.4–16.8 µm (E = 2.54–6.13; n = 6/1). Stipitipellis a cutis. caulocystidia and hymenial clusters absent. Oleiferous hyphae, Lipoid Globules and Brilliant granules absent in the trama; basidia with abundant granules. Pigmentation colorless in the pileipellis, parietal in the entangled layer. Clamp connections present in all tissues.

Ecology & distribution — Solitary in leaf humus, Mossman and Daintree National Parks, far Northern Queensland, mid-March.

Additional collections examined — **AUSTRALIA. Queensland**, Cook Region, Daintree National Park, Tribulation section, Emmagen Creek Track, 16°02′19.7″S 145°27′41.7″E, 15 March 2010, DL Largent 9783.

Distinctive characters — Tricholomatoid basidiomata, subfelty brown pileus, lamellae with droplets drying as reddish brown spots, unpleasant taste and odor, and a pileipellis as an anticlinal layer of entangled slender colorless hyphae with narrow 3–5 µm wide terminal cells.

Comments — Phylogenetically *Entoloma guttulatum* clusters with *E. kewarra, E. pamelae,* and *E. illinitum* (from Guyana) but few morphological features

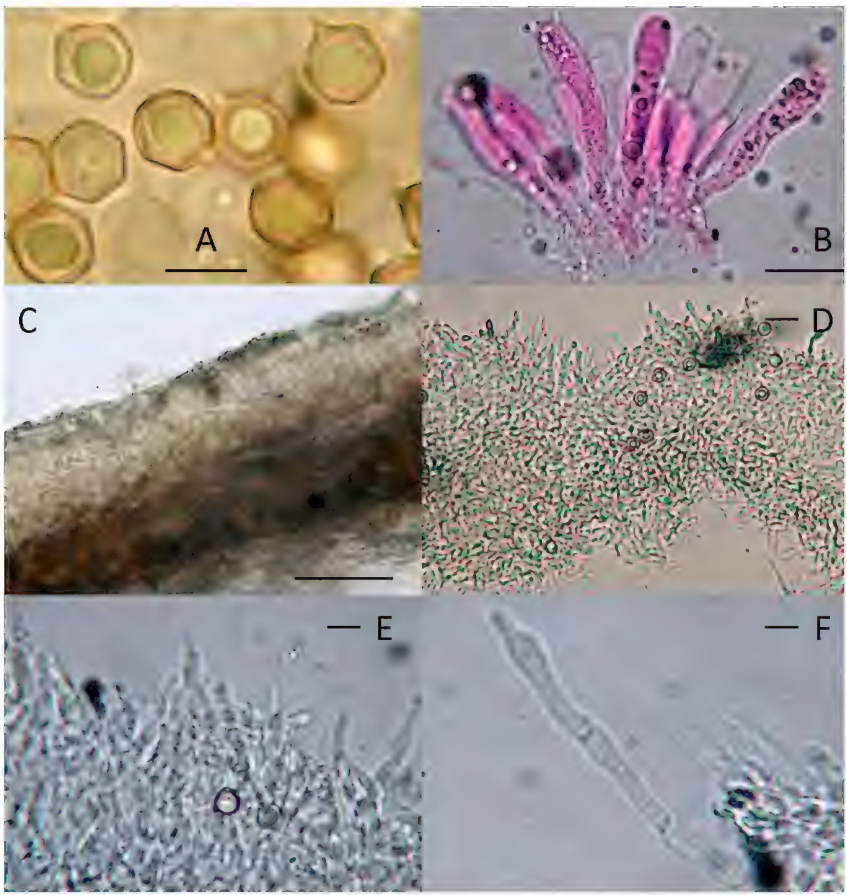


Plate 11. *Entoloma guttulatum* (DLL9791 holotype). A. Basidiospores. B. Basidia, basidioles. C. Pileipellis (radial section at pileus disc). D. Pileipellis (squash mount) E. Pileipellis (squash mount) F. Pileocystidium. Bars: A = 8 µm; B = 20 µm; C = 25 µm; D = 35 µm; E = 10 µm; F = 5 µm.

are shared between species. For example, the basidiospores are very small and distinctly angular in *E. kewarra* and *E. pamelae*, small and obscurely angular in *E. illinitum*, and large and distinctly angular in *E. guttulatum*.

Entoloma pingue Corner & E. Horak (from a *Casuarina* forest in the Solomon Islands), which shares a large fleshy stature, decurrent lamellae, and isodiametric basidiospores (7–9 µm diam.) with *E. guttulatum,* can be distinguished by its pale fuscous pileus, stipe changing to pale brown, white lamellae without reddish spots, 30-40 µm long basidia, and pigmented pileipellis (Horak 1980).

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Leptocorticium indicum sp. nov. from India

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Abstract — A new corticioid species, *Leptocorticium indicum,* is described on a decaying log of *Rhododendron arboreum* from Uttarakhand state in India.

Key words — *Agaricomycetes*, Tehri Garhwal, Kaddukhal

While conducting fungal forays in Kaddukhal area of district Tehri Garhwal, Uttarakhand, India, Samita and Sanyal collected an unknown corticioid fungus on a decaying log of *Rhododendron arboreum.* On the basis of macroscopic and microscopic features and comparison with available literature (Hjortstam & Ryvarden 2002, Nakasone 2005, Bernicchia & Gorjón 2010, Gorjón & Saitta 2014), we found the species different but closely related to the known species of *Leptocorticium*, hence the description of a new species in this genus. The material was also analyzed by Prof. Nils Hallenberg, who supported the concept of a new species within *Leptocorticium.*

Leptocorticium indicum Samita, Sanyal & Dhingra, **sp. nov.** Plates 1, 2

MycoBank 808626

Differs from *Leptocorticium tenellum* in having larger basidia, smaller broadly ellipsoid to ovate to subglobose basidiospores, and presence of gloeocystidia and from *L. gloeocystidiatum* in having broadly ellipsoid to ovate to subglobose basidiospores.

Type: India, Uttarakhand, Tehri Garhwal, Kaddukhal, on decaying log of *Rhododendron arboreum* Sm. (*Ericaceae*), 20 August 2010, Samita 6092 (PUN, **holotype**).

Etymology: The epithet refers to the country of the type collection.

Basidiocarp resupinate, effused, adnate, ≤80 µm thick in section; hymenial surface smooth, grayish orange; margins thinning, fibrillose, paler concolorous, or indeterminate. Hyphal system monomitic. Generative hyphae ≤4.5 µm wide, septate, clamped, thin- to thick-walled, with or without oily contents;

Plate 1. *Leptocorticium indicum* (holotype). Basidiocarp.

basal hyphae parallel to substrate, less branched; subhymenial hyphae vertical, more densely branched. Dendrohyphidia abundant, irregularly branched, non-dextrinoid, with thin to slightly thickened walls. Gloeocystidia 61–72 × 7.5–9 µm, flexuous, with round to moniliform tips, embedded to projecting ≤40 µm out of hymenium, with basal clamp and oily contents negative to sulphovanillin. Basidia 41–51 × 6.6–7.7 µm, clavate, 4-sterigmate, with basal clamp, with or without oily contents; sterigmata ≤7.5 µm long. Basidiospores 5.5–6.6 × 3.3–4.4 µm, broadly ellipsoid to ovate to subglobose, thin-walled, inamyloid, acyanophilous.

Remarks— This species is placed in *Leptocorticium* based on the presence of a thin and poorly developed subiculum, catahymenium with abundant dendrohyphidia, flexuous gloeocystidia, and clavate basidia. *Leptocorticium tenellum* Nakasone differs from *L. indicum* by its larger, cylindrical to ellipsoid to sub-fusiform basidiospores (7–9.5 × 3–5 µm), absence of gloeocystidia, and smaller, usually utriform basidia (15–26 × 4.5–8 µm; Nakasone 2005). *Leptocorticium gloeocystidiatum* Gorjón & Saitta differs from *L. indicum* by its smaller, ellipsoid basidiospores (4–5 × 2.5–3 µm) and smaller basidia (15–20 × 3–4 µm; Gorjón & Saitta 2014).

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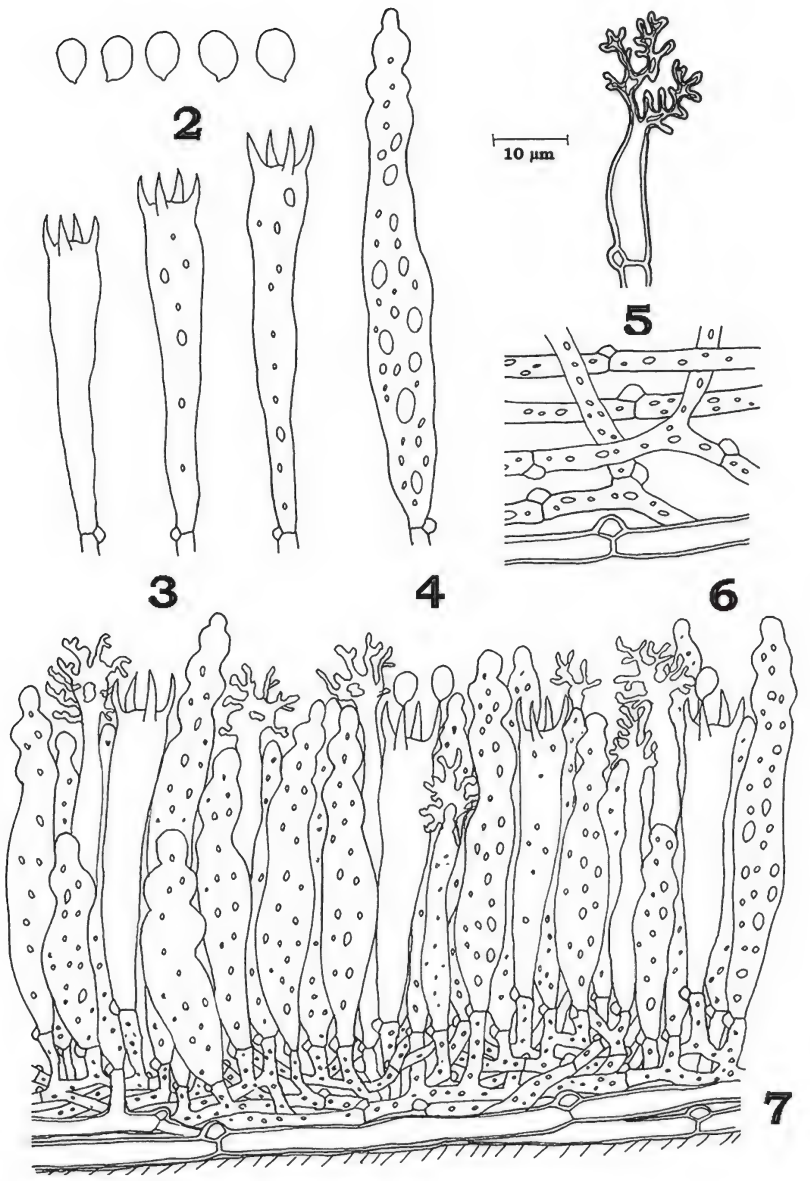

Plate 2. *Leptocorticium indicum* (holotype).
1. Basidiospores. 2. Basidia. 3. Cystidium. 4. Dendrohyphidium.
5. Hyphae. 6. Vertical section through basidiocarp.

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A new species of *Podosphaera* sect. *Sphaerotheca* from China

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Abstract — *Podosphaera girardiniae* (*Erysiphaceae*, *Podosphaera* sect. *Sphaerotheca*), identified in China on *Girardinia suborbiculata*, is described, illustrated, and compared with allied species. The phylogeny of this new species has been inferred from internal transcribed spacer (ITS) and 28S rDNA sequence analyses.

Key words — molecular phylogeny, morphology, taxonomy

Introduction

In the autumn of 2013, *Girardinia suborbiculata* (*Urticaceae*) was identified in Shaanxi, China, that was heavily infected by a powdery mildew. Based on the asexual morph characterized by catenescent conidia with distinct fibrosin bodies and chasmothecia with a single ascus and mycelioid appendages, this collection was easily assignable to *Podosphaera* sect. *Sphaerotheca* (Lév.) de Bary, as currently circumscribed and recognized (Braun & Cook 2012). This section consists of 75 species, with 57% of the host plants belonging to the *Rosaceae* (Takamatsu et al. 2010). Given the relatively large peridial cells, the powdery mildew on *Girardinia* can be placed in *Podosphaera* subsect. *Magnicellulatae* (U. Braun) U. Braun & Shishkoff (Braun & Cook 2012).

Podosphaera parietariae (Schwarzman) U. Braun & S. Takam. on *Parietaria* spp. in Asia and Europe is the only species on an urticaceous host assigned to *Podosphaera* sect. *Sphaerotheca,* where it has been placed based on its small (8–25 µm diam.) peridial cells (Braun 1987, Braun & Takamatsu 2000, Braun & Cook 2012). Its morphology easily eliminates *P. parietariae* as the causal agent of the *Girardinia* infection in China. However, in subsect. *Magnicellulatae,* plurivorous races must be taken into consideration, particularly the *P. xanthii* (Castagne) U. Braun & Shishkoff complex to which the collection on *Girardinia*

appears to belong. Therefore, it was necessary to perform molecular sequence analyses to elucidate the taxonomic status of the powdery mildew involved.

Materials & methods

Living leaves of *Girardinia suborbiculata* bearing the holomorph of a powdery mildew were collected in October 2013 in the Qinling Mountains within Lueyang County in China. Herbarium specimens were deposited in the Mycological Herbarium of Forestry College, Northwest A & F University, Yangling, Shaanxi Province, China (HMNWAFU-CF) and the herbarium of Martin Luther University, Halle (Saale), Germany (HAL).

The specimen was mounted in distilled water and examined using light microscopy (Olympus, CX31RTSF, Japan). The teleomorphic features of the fungus, including chasmothecia, appendages, asci, and ascospores, were described, measured, and photographed. A scanning electron microscope (JEOL, JSM-6360LV) was used to observe the anamorph ultrastructure, particularly the surface features of conidia (Cook et al. 1997) and appressoria of this fungus; SEM images were taken.

Genomic DNA was extracted from chasmothecia using Chelex-100 (Walsh et al. 1991; Hirata & Takamatsu 1996). The ITS region of the nuclear rDNA (including 5.8S and 28S rDNA sequences with domains D1 and D2) were amplified via polymerase chain reaction (PCR) using primers designed for each region: ITS1 and ITS4 (White et al. 1990) were used to amplify the ITS region, while LSU1 and LSU2 (Scholin et al. 1999) were used to amplify the 28S rDNA sequence.

The PCR assays were conducted in a 50 µL final volume (Hirata & Takamatsu 1996) containing 27 µL of 2× BoisTaq PCR MasterMix, 1 µL of each of primer, 1 µL of the extracted DNA and 20 µL of ddH2O (Hirata & Takamatsu 1996). Thermal cycling in a PTC-200 thermal cycler (BioRad) comprised an initial denaturation step at 95°C for 5 min, 35 cycles of 94°C for 1 min + 60°C for 1 min + 72°C for 1 min, and a final elongation step at 72°C for 8 min. A negative control for each set of reactions replaced template DNA with ddH_2O. The PCR products were separated by electrophoresis on a 2% agarose gel in TAE buffer and purified using the Zymoclean™ Gel DNA Recovery Kit, according to the manufacturer's instructions. The purified DNA products were ligated into the pMD18-T vector (Takara) and transformed into *E. coli* DH5α cells. The cloned fragments were sequenced by Sangon Biotech (Shanghai) Co., Ltd.

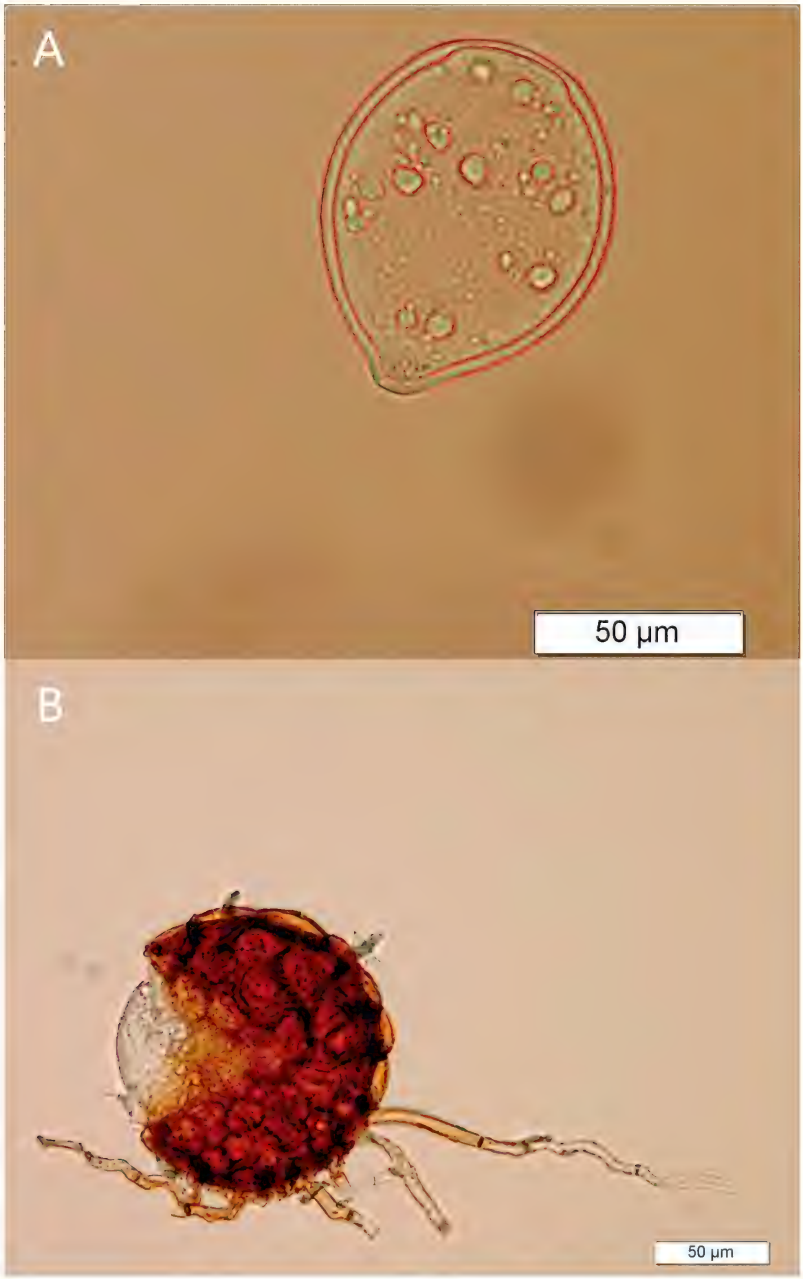
All DNA sequences were aligned using Clustal X 1.81 (Thompson et al. 1997), and the alignments were adjusted following Nei & Kumar (2000). All positions containing gaps or missing data were eliminated from the dataset. Cladistic trees were constructed using the neighbor-joining method with the Kimura 2-parameter substitution model in MEGA 4.0 (Tamura et al. 2007). Branch robustness was assessed by bootstrap analysis with 1,000 replicates.

Taxonomy

Podosphaera girardiniae Z.M. Cao & L.C. Bai, **sp. nov.** PLATES 1–3

MYCOBANK MB 810810

Morphologically and phylogenetically similar to *Podosphaera xanthii* but sufficiently distinct genetically to be considered a separate species.



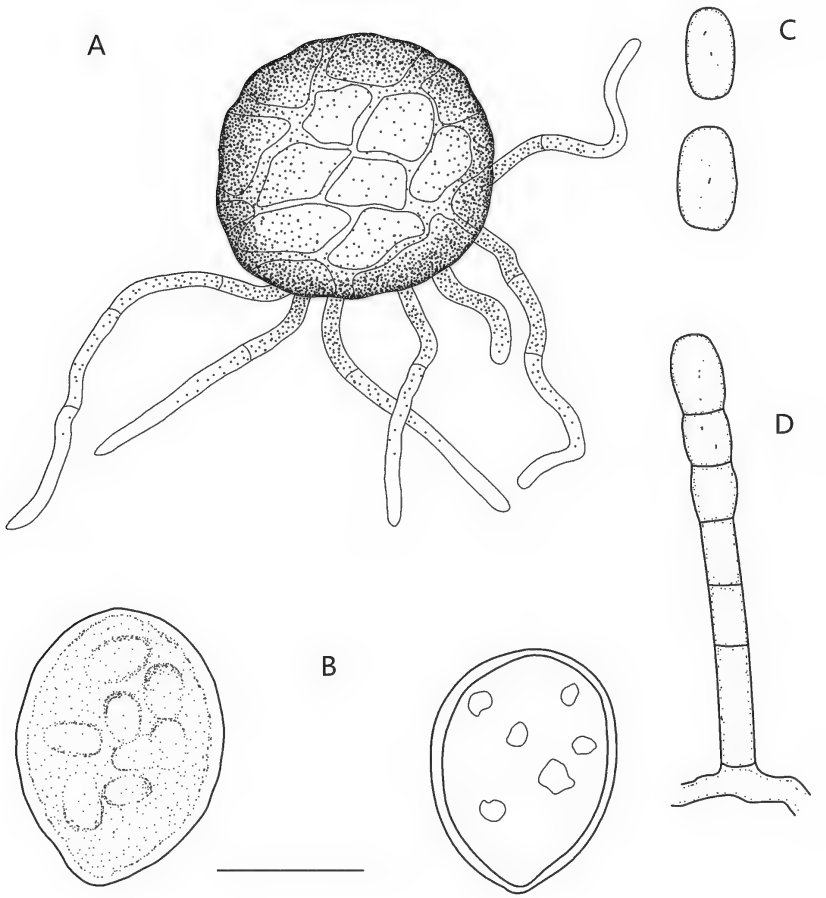
Pl. 1. *Podosphaera girardiniae* (Holotype). A. asci; B. chasmothecia and appendages.

Pl. 2. *Podosphaera girardiniae* (Holotype). A. conidia and conidiophore; B. conidium; C. conidiophore, conidia, and hyphae. Scale bars: A, B = 10 μm; C = 50 μm.

Type: China, Shaanxi, Qinling Mountains, Lueyang County, 33°19′29″N 106°08′29″E; alt. 840 m, on living leaves of *Girardinia suborbiculata* C.J. Chen (*Urticaceae*), Oct. 2013, L.C. Bai (**Holotype**, HMNWAFU-CF 2013125; **isotype**, HAL 2650 F; GenBank KJ540945, KJ540944).

Etymology: referring to the host genus.

Mycelium mainly foliicolous, amphigenous, effuse or in irregular patches, later confluent, often covering the entire leaf surface, white, persistent. Hyphal appressoria indistinct to slightly nipple-shaped. Hyphae smooth or almost smooth, 3–8 μm wide, colorless. Conidiophores arising from the upper surface of hyphal mother cells, erect, straight to somewhat curved, foot-cells cylindrical, about 34–76(–92) × 9–15 μm, followed by 1–3 shorter cells, forming catenescent conidia. Conidia ellipsoid-ovoid, doliiform, 25–36 × 15–21 μm, with a length/width ratio of 1.4–2.1. Chasmothecia mainly epiphyllous, gregarious, 82–133 μm diam. , subglobose; peridium cells conspicuous, large, irregularly shaped, 14–44 μm diam, appendages usually few, about 2–14, in the lower half, mycelioid, simple, unbranched, sometimes interlaced with each other, about 0.4–3.9 times as long as the chasmothecial diam. (up to about 410μm), 5–9 μm wide, brown below and paler towards the tip or most of the short appendages brown throughout, 0–4-septate, walls thin, smooth or almost so; ascus broadly ovoid or broadly ellipsoid-subglobose, 71–96 × 61–77 μm, almost sessile, terminal oculus 15–22 μm diam., wall 1.5–3 μm thick,



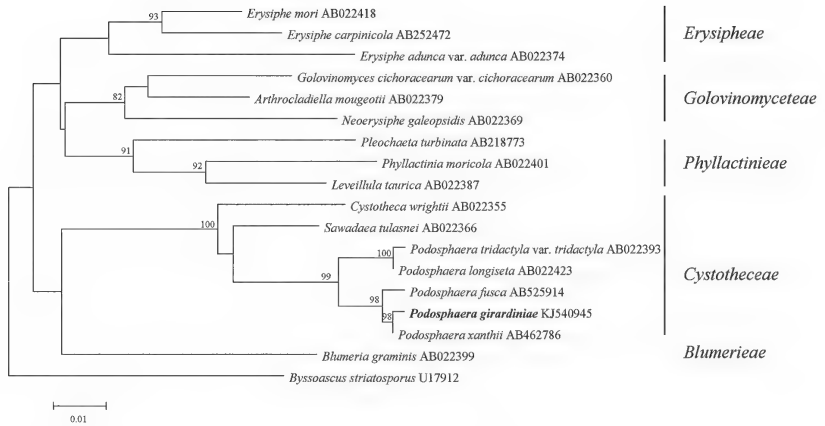
Pl. 3. *Podosphaera girardiniae* (Holotype). A. chasmothecia and appendages; B. asci; C. conidia; D. conidia and conidiophore. Scale bars = 50 µm.

6–8-spored; ascospores broadly ellipsoid-ovoid, 16–23 × 13–17 µm, colorless, development relatively late.

Phylogeny

28S analysis Pl. 4

The 28S rDNA sequence comprised 632 total characters and was deposited in GenBank under accession number KJ540945. The sequence was aligned



Pl. 4. Neighbor-joining tree based on distances derived from sequences of the 28S rRNA genes from 17 taxa of *Erysiphaceae,* with *Byssoascus striatosporus* as outgroup. The bar indicates a distance of 0.01.

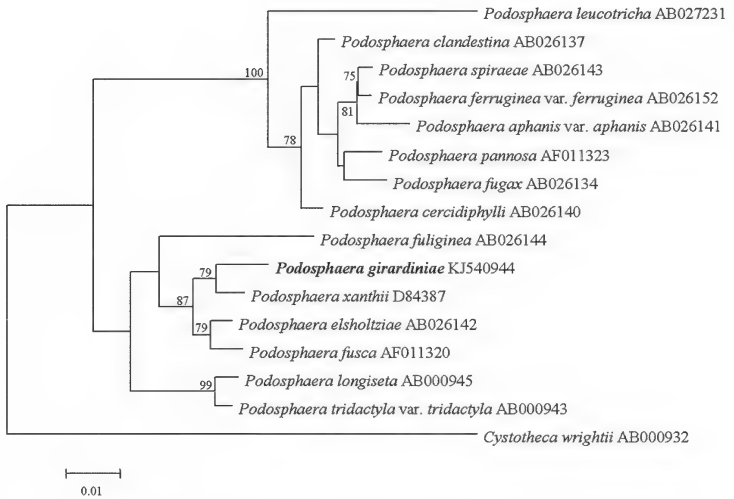
with 15 sequences representing the five tribes of *Erysiphaceae*. The 28S rDNA phylogenetic tree places the *Podosphaera girardiniae* sequence in a strongly supported clade (bootstrap value = 98%) where it clusters with *P. xanthii*, clearly supporting its placement in the genus *Podosphaera*, probably within subsect. *Magnicellulatae*. *Byssoascus striatosporus* (G.L. Barron & C. Booth) Arx was used as outgroup.

ITS analysis Pl. 5

The ITS rDNA sequence analysis comprised 499 total characters and was deposited in GenBank under accession number KJ540944. The sequences were aligned with 14 sequences representing *Podosphaera* Kunze emend. U. Braun & S. Takam. *Cystotheca wrightii* Berk. & M.A. Curtis was used as outgroup. The ITS phylogenetic tree placed *Podosphaera xanthii* and *P. girardiniae* in one clade with 79% bootstrap support. The *P. girardiniae* sequences differ from all available sequences representing other *P.* sect. *Sphaerotheca* species, including *P. xanthii*. The phylogenetic analyses of the rDNA sequences indicated that *Podosphaera* on *Girardinia suborbiculata* represents a new species.

Discussion

The only other *Podosphaera* known on an urticaceous host, *P. parietariae*, clearly differs morphologically from *P. girardiniae*, and its chasmothecia



Pl. 5. Neighbor-joining tree based on distances derived from the ITS1, ITS2, and 5.8S rRNA gene sequences from 15 *Podosphaera* taxa, with *Cystotheca wrightii* as outgroup. The bar indicates a distance of 0.01.

with small peridial cells place it in subsect. *Sphaerotheca*. Our phylogenetic analyses support a close affinity of *P. girardiniae* with *P. xanthii*, from which it is morphologically barely distinguishable. Nonetheless the genetic differences are too significant to assign the *Girardinia* powdery mildew to *P. xanthii*. The sequence selected for *P. xanthii* was previously used by other authors (e.g., Kousik et al. (2011), who demonstrated that this sequence and other sequences of *P. xanthii* from several cucurbits, beans and exotic impatiens species share 100% ITS similarity). These genetic differences support the *Girardinia* powdery mildew as a new species.

Acknowledgments

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Rhizoglomus, a new genus of the *Glomeraceae*

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Abstract — *Rhizoglomus* gen. nov. (*Glomeraceae*, *Glomeromycetes*) is proposed, typified by *Glomus intraradices* [≡ *Rhizoglomus intraradices*]. The genus encompasses species of arbuscular mycorrhizal fungi that frequently form abundant spores in soil and roots and is morphologically characterized by spores with cylindrical subtending hyphae (usually with an open pore at the base) and at least two or three (rarely up to five) distinct wall layers. Phylogenetically, the genus forms a separate clade in the *Glomeraceae*. In addition to *R. intraradices*, the genus includes *R. aggregatum, R. antarcticum, R. arabicum, R. clarum, R. custos, R. fasciculatum, R. invermaium, R. irregulare, R. manihotis, R. microaggregatum, R. natalense,* and *R. proliferum*. Some of these species were previously assigned to *Rhizophagus* (type: *R. populinus*), a pathogenic genus that does not belong in the *Glomeromycota*.

Key words — *Glomerales*, *Glomus aggregatum*, *Glomus clarum*, *Glomus fasciculatum*, *Glomus irregulare*

Introduction

Glomus Tul. & C. Tul. s.l. (*Glomeraceae*, *Glomeromycetes*) comprises a heterogeneous assembly of arbuscular mycorrhizal (AM) fungi (e.g., Morton & Benny 1990, Schwarzott et al. 2001). In an attempt to redefine the genus more naturally, Schüßler & Walker (2010) reassigned several *Glomus* species characterized by the frequent formation of intraradical spores to *Rhizophagus* P.A. Dang. Dangeard (1896), who identified *Rhizophagus populinus* as the causal agent of a severe root disease of poplar (*Populus* spp.) in northern France,

later described the root infection structures of the species (Dangeard 1900) but neither designated nor deposited type specimens, as this was not the procedure at that time. Consequently, the vague type description (Dangeard 1896) and detailed drawings (Dangeard 1900) remain the only reference for *Rhizophagus* and its type species, *R. populinus*.

Several decades later, Gerdemann & Trappe (1974) synonymized *Rhizophagus* with *Glomus*. Later, reviewing the type description (Dangeard 1896), Schüßler & Walker (2010) noted, "examination of the protologue of *R. populinus* reveals that the species is an arbuscular mycorrhizal fungus that produces abundant spores in roots." For this reason they resurrected *Rhizophagus,* placing the genus in the *Glomeraceae*. Here, we argue that the name *Rhizophagus* should not be assigned to any species in the *Glomeromycota*.

First: Dangeard (1896) described (without providing any illustrations) a disease of poplar roots occurring under wet soil conditions, which produced "sporangia" and finely ramified hyphae inside the root epidermal cells. The morphology he observed led him to propose that the organism represented a new genus in the *Chytridineae*. He related the death of the poplar trees to this pathogen and not to a foliar disease. He did not observe germination of the "sporangia" but explained that there were durable spores or cysts in the root cells with a thick membrane and with protoplasm containing abundant oil. Dangeard (1896) did not describe that numerous spores (or sporangia or cysts) were formed in the infected poplar roots. The diagnosis of *Rhizophagus* therefore, published by Schüßler & Walker (2010), does not correspond with the observations described by Dangeard (1896).

Second: Dangeard (1900), through illustrations, showed that the infection structures of *R. populinus* in poplar roots comprised two structural types: i) those resembling vesicles (which he referred to as sporangia with dense protoplast) and ii) arbuscules (termed ramified hyphae in cells) of mycorrhizal root colonization. He did not recognize these structures as endomycorrhizae, however. He also illustrated spores, which were not connected to the vesicles or arbuscules. These spores were spherical, small (in relation to the root cells), and with an apparently single wall layer and one or two hyphal connections formed terminally or intercalary (see plate IX, D, E). Dangeard (1900) called these cells 'cysts' ('kystes' in French) because they contained a lipid globule. Recognizing these structures as characteristic of chytrids, Dangeard (1900) assigned *R. populinus* to the *Chytridineae*, a suborder containing root pathogenic fungi.

Third: Saccardo & Trotter (1912) transferred *Rhizophagus* to the *Peronosporaceae*[1] and stated that "sporae perdurantes intercalares v. terminales,

[1] In 1912 mycologists placed root-borne and root disease-causing fungi such as *Pythium* into the *Peronosporaceae* (see Middleton 1952, for historic overview). Today *Peronosporaceae* contain only foliar disease-causing fungi.

globosae, membrana levi et guttulis pluribus praeditae." Here, reference is clearly made to the lipid globules in the globose spores (also drawn by Dangeard (1900) in plate IX, Figs. D&E), a characteristic feature for spores of some representatives of the *Chytridiales* and *Oomycetes* (Hawksworth et al. 1983). Saccardo & Trotter (1912) referred to *R. populinus:* "Characteres generis. Hab. in radicibus junioribus Populi in Gallia occid. – Profunde penetrat et plantas necat," which clearly states that *R. populinus* causes death of the host plants. It must be noted, however, that early in the 20th century, fungal root pathogens were separated from *Peronosporaceae* and placed in families such as *Pythiaceae* (Middleton 1952). To our knowledge, no one ever officially transferred *Rhizophagus* to *Pythiaceae* or any other family in the *Oomycetes*.

Fourth: Petri (1919) argued that the structures depicted by Dangeard (1900) in poplar tree roots belonged to the endomycorrhizae and were vesicles and arbuscules, not the cause of death of the poplar trees. However, Petri ignored the cysts with lipid globules illustrated by Dangeard (1900). He believed that the poplar tree deaths in Northern France were resulted from a foliar disease and that *R. populinus* was not causing root disease.

Fifth: Butler (1939), who reviewed vesicular-arbuscular mycorrhizal fungi, showed that extramatrical fruiting bodies of *Endogonaceae* (including some species currently assigned to *Glomeraceae*) were connected with internal structures resembling those depicted by Dangeard (1900) that were later identified as vesicles and arbuscules. Butler concluded that the vesicles and arbuscules formed in roots represented the "imperfect form" of *Endogonaceae,* a form named by that Dangeard (1900) as *R. populinus*. His statement had far-reaching consequences: for a decade *Rhizophagus* was considered the most widely distributed fungus in the world, until Mosse (1953) successfully isolated the mycorrhizal spores and vesicular-arbuscular mycorrhizal structures from roots during inoculation experiments. Mosse (1953, 1970) showed that different spore "types" (i.e., species), such as "yellow vacuolated" and "honey-colored" spores, formed arbuscules and vesicles in roots.

Sixth: Gerdemann (1968) concluded, "...there is little reason to continue to use the name *Rhizophagus* [for a fungus forming vesicles and arbuscules in roots] ... as the genus *Endogone* was described much earlier." It should be noted that by 1968 vesicular-arbuscular mycorrhiza-forming fungi were organized in *Endogone* (*Endogonaceae*). A few years later Gerdemann & Trappe (1974) separated many species from the *Endogonaceae,* placing them into the genus *Glomus,* earlier described by Tulasne & Tulasne (1844). Following Gerdemann's (1968) reflections, the vesicles and arbuscules depicted by Dangeard (1900) could not be a morphological character restricted to *Rhizophagus,* as *Glomus, Sclerocystis* (described by Berkeley & Broome in 1873), and most other glomeromycete genera form such mycorrhizal structures.

Seventh: Gerdemann & Trappe (1974) synonymized *Rhizophagus* with *Glomus* without providing a specific justification, which is intriguing, given that Gerdemann (1968) had earlier doubted the validity of applying the name *R. populinus* to a vesicular-arbuscular fungal species. Indeed, at this point Gerdemann & Trappe (1974) should have eliminated the name *Rhizophagus* from the *Endogonaceae*.

Although Dangeard (1896, 1900) interpreted vesicles of a vesicular-arbuscular mycorrhizal fungus in poplar roots as sporangia, it is now clear that this was incorrect and that the spores (cysts) he described were not, in fact, released from these sporangia. This observation was also made after Schlicht (1889) had already described AM type endomycorrhizae and determined that numerous fungal species could be involved. It has since been established that AM vesicles and arbuscules are so morphologically uniform that they cannot be used to differentiate species, genera, families, or orders of in the *Glomeromycota* (Oehl et al. 2011a,b,c). Further, molecular techniques have demonstrated that single roots can be colonized simultaneously by numerous glomeromycete species (e.g., Lekberg et al. 2007, Tchabi et al. 2009, Borriello et al. 2012, Wetzel et al. 2014). Consequently, we assume that the vesicle and arbuscule structures delineated by Dangeard (1900) most likely resulted from combined infections of a number of species representing different orders of the *Glomeromycota*.

It is well established that poplar trees host non-pathogenic vesicular-arbuscular mycorrhizal fungi (Clark 1963), and it is likely that Dangeard (1900) observed such vesicles and arbuscules in poplar root cells in addition to spores of a disease-causing fungus such as *R. populinus*. Dehne (1982) and Linderman (1994) state that fungal root pathogens can occupy root cortical cells adjacent to those colonized by AM fungi, and that mycorrhizal colonization of a root can never confer complete immunity against any root disease (see Bagyaraj 1984). Such colonization by AM fungi in close vicinity of pathogens such as *Pythium* or *Rhizoctonia* has frequently been reported (e.g., Dehne 1982, Linderman 1994).

Additionally confusing, with respect to *Rhizophagus* and its type species, is that to date, no vesicular-arbuscular mycorrhiza-forming species of the *Glomeromycota* has been recorded as a root pathogen that kills a plant. This would represent a fundamental change to the current standing of vesicular-arbuscular mycorrhiza as the most widespread mutualistic symbiosis between flowering plants and soil-borne microorganisms.

It is actually quite remarkable that researchers and taxonomists have paid attention to only the vesicle and arbuscule structures described by Dangeard (1900) while after 1912 ignoring or overlooking the cysts he described (Dangeard 1896, 1900). According to our historical analysis, Dangeard (1900) described the real species in the last paragraph: "The cysts of *Rhizophagus populinus* are

spherical; the content is oily (P. IX, E); some even contain a large drop of oil (P. IX, D)." Saccardo & Trotter (1912) identified these cysts (spores) as the causal agent of a mortal poplar disease. Thus the actual *Rhizophagus populinus* cannot belong to the *Glomeromycota*, but rather represents *Oomycetes*.

We therefore conclude that the genus name *Rhizophagus* cannot be applied to a group of glomeromycotan fungi that form spores in plant roots, as suggested by Schüßler & Walker (2010). Hence, we propose a new genus, *Rhizoglomus*, to accommodate vesicular-arbuscular mycorrhizal fungal species of the *Glomeraceae* that often form abundant spores in the soil and roots of vascular plants.

Materials & methods

Specimens and original species descriptions of ten *Glomus* spp., one *Endogone* sp., and two recently described *Rhizophagus* spp. (Symanczik et al. 2014, Błaszkowski et al. 2014) were re-examined. Type specimens, ex-type material (specimens or single species cultures either separated or propagated from original type material, and deposited at a herbarium or collection that was not mentioned in the protologue), and non-type specimens were provided by the herbaria OSC (Corvallis, Oregon), Z+ZT (Zurich), DEPE (Szczecin, Poland), URM (Recife, Brazil), Culture Collection of Vesicular-Arbuscular Mycorrhizal Fungi (INVAM), Swiss collection of Arbuscular mycorrhizal Fungi (SAF, Agroscope), and the private AM fungal collections of Sieverding, Oehl, and Błaszkowski. The Hall & Abbott (1979) photographic slide collection was also reviewed. For this paper, all original species descriptions and published species emendations were also considered.

Specimens were either mounted in lactophenol, in polyvinyl alcohol-lactic acid-glycerol (PVLG), in Melzer's reagent, in a mixture of PVLG + Melzer's reagent (1:1; Brundrett et al. 1994), in a mixture of lactic acid to water (1:1), or in water. When available, spores freshly isolated from soils or bait cultures were also mounted and analyzed. Spore wall terminology follows the nomenclature of Oehl et al. (2005, 2011b). Spore walls, germination structures, and all other mycorrhizal structures were investigated using a compound microscope at 100–1000× magnifications. All spore observations and all information on spore characteristics are based on spores extracted from soil, bait cultures, or single species cultures. No information is provided from in vitro-cultured material.

Results

Taxonomic analyses

Rhizoglomus Sieverd., G.A. Silva & Oehl, **gen. nov.** FIGS 1–15

MYCOBANK MB 803191

Differs from *Glomus* by regularly open pores at the spore bases, and usually wide open pore channels within the frequently cylindrical subtending hyphae.

TYPE SPECIES: *Glomus intraradices* N.C. Schenck & G.S. Sm. [≡ *Rhizoglomus intraradices*].

Etymology: from Latin: *rhiza* = root, referring to the often abundant hyphae, arbuscules, vesicles and spores formed in roots; and *Glomus*, referring to the genus in which the type species of *Rhizoglomus* was first described.

Spores formed in loose sporocarps, in clusters, or singly in soil, and frequently in roots. Wall of the subtending hyphae continuous with the spore wall, and for a certain distance concolorous with the spore wall, or slightly lighter in color. Subtending hypha cylindrical or seldom slightly funnel shaped at spore base. Pore at spore base regularly open, rarely closed by a septum. Spore walls show more than one, and generally two to three (and up to five) distinct layers, of which one or several of the outermost may separate when pressure is applied to spores. Forming vesicular-arbuscular mycorrhizae, whose fungal structures stain blue to dark blue in trypan blue.

New combinations

Rhizoglomus aggregatum (N.C. Schenck & G.S. Sm.) Sieverd., G.A. Silva & Oehl **comb. nov.**

MycoBank MB 803193

≡ *Glomus aggregatum* N.C. Schenck & G.S. Sm., Mycologia 74: 80. 1982.

Specimen examined: Type described from a pot culture by Schenck & Smith (1982). Isolated from Florida State near Haines City. Holotype OSC #40255, examined by Oehl at OSC in 2002.

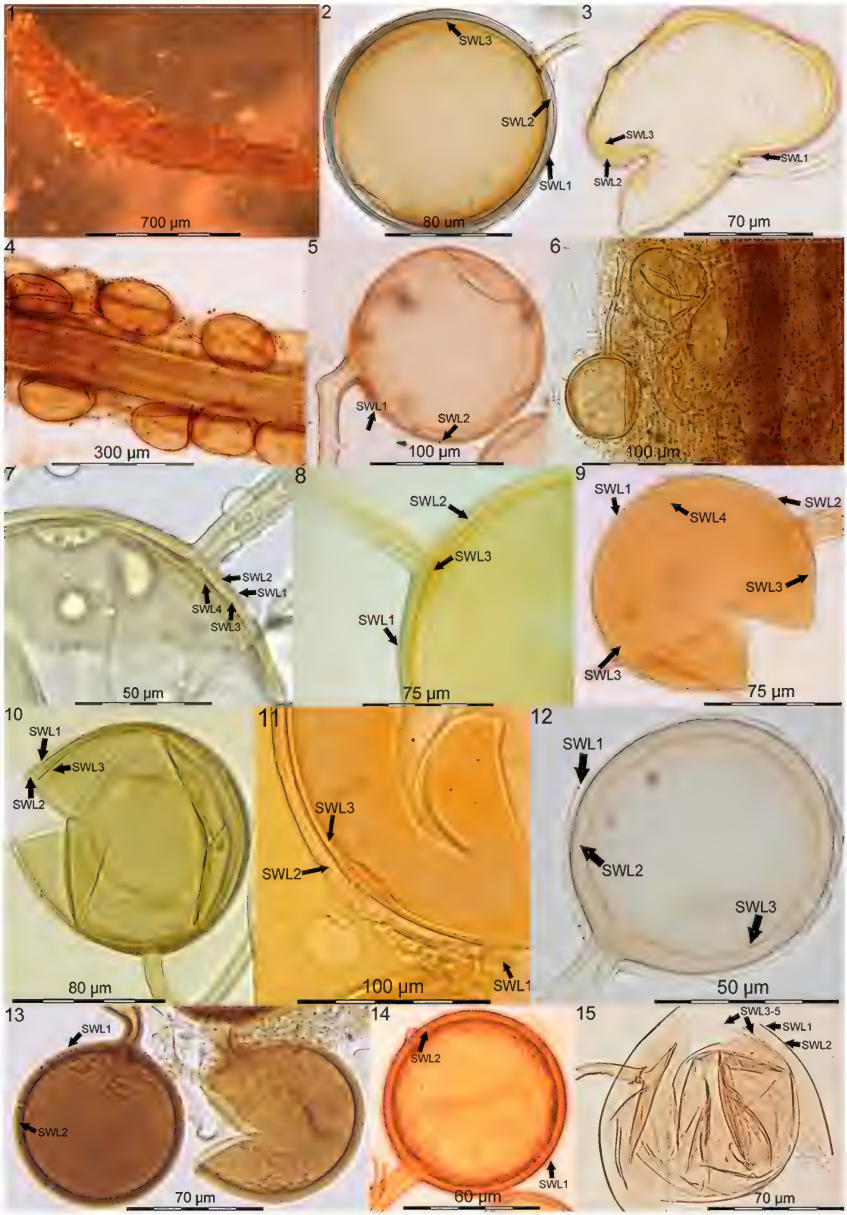
Rhizoglomus antarcticum (Cabello) Sieverd., G.A. Silva & Oehl **comb. nov.**

MycoBank MB 803194

≡ *Glomus antarcticum* Cabello, Mycotaxon 51: 124. 1994.

Specimen examined: Type described from rhizospheric soil of *Dechampsia antarctica* at Danco Coast, Base Primavera, Antarctic Peninsula (Argentina), by Cabello et al. (1984). Specimen was loaned by M. Cabello to B.T. Goto (UFPE, Recife, Brazil) in 2009 and examined by Oehl at URM.

Figs 1–12. Spores of ***Rhizoglomus* spp.** from soils or within roots. ***Rhizoglomus intraradices*** (from Germany, ZT Myc 30479): **1.** Spores formed within the host root, isolated from pot culture. **2.** Spore with three wall layers (SWL1–3). ***Rhizoglomus irregulare*** (from Switzerland, ZT Myc 30483): **3.** Spore with three wall layers (SWL1–3). ***Rhizoglomus aggregatum*** (type, OSC): **4.** Spores formed within the host root. **5.** Spore with two wall layers (SWL1–2). ***Rhizoglomus microaggregatum*** (type, OSC): **6.** Spores formed within the host root; mature spores have two layers. ***Rhizoglomus proliferum*** (ex type, ZT Myc 30484): **7.** Spore with four wall layers (SWL1–4). ***Rhizoglomus clarum*** (from an INVAM culture): **8.** Spore with three wall layers (SWL1–3). ***Rhizoglomus natalense*** (from Natal, Brazil, isotype, DEPE 3384): **9.** Spore with four wall layers (SWL1–4). ***Rhizoglomus custos*** (ex type, ZT Myc 49021): **10.** Spore with three (SWL1–3) of four wall layers described visible. ***Rhizoglomus manihotis*** (from type, OSC): **11.** Spore with evanescent outer wall layer (SWL1), a rather thick hyaline layer (SWL2) and up to two separable yellow wall layers (SWL3). ***Rhizoglomus fasciculatum*** (from Switzerland, ZT Myc 30478): **12.** Spore with three wall layers (SWL1–3) and open pore. ***Rhizoglomus invermaium*** (isotype, OSC): **13.** Spores with two wall layers (SWL1–2).



Rhizoglomus antarcticum (from type, M. Cabello collection): **14.** Spore with two (SWL1–2) layers visible (of three described). ***Rhizoglomus arabicum*** (isotype, DEPE): **15.** Spore with 2–5 wall layers, depending on layer and laminae definition, not easily discerned.

Rhizoglomus arabicum (Błaszk., Symanczik & Al-Yahya'ei) Sieverd., G.A. Silva & Oehl **comb. nov.**

MycoBank MB 809436

≡ *Rhizophagus arabicus* Błaszk., Symanczik & Al-Yahya'ei, Mycologia 106: 253. 2014.

Specimen examined: Type described from two pot cultures by Symanczik et al. (2014). Isolated from an undisturbed natural field at Al-Kamel in Al-Sharqyia region of Oman; examined by Oehl in 2014. One slide deposited at Z+ZT (ZT Myc 57169).

Rhizoglomus clarum (T.H. Nicolson & N.C. Schenck) Sieverd., G.A. Silva & Oehl **comb. nov.**

MycoBank MB 803195

≡ *Glomus clarum* T.H. Nicolson & N.C. Schenck, Mycologia 71: 182. 1979.

≡ *Rhizophagus clarus* (T.H. Nicolson & N.C. Schenck) C. Walker & A. Schüssler, The *Glomeromycota*: 19. 2010.

Specimen examined: Type described from a pot culture by Nicolson & Schenck (1979) from soil on the Agronomy Farm of the University of Florida. Type at OSC, examined by Oehl at OSC in 2002, and by Sieverding at Centro Internacional de Agricultura Tropical (CIAT; Cali, Colombia) culture collection.

Rhizoglomus custos (C. Cano & Dalpé) Sieverd., G.A. Silva & Oehl **comb. nov.**

MycoBank MB 803196

≡ *Glomus custos* C. Cano & Dalpé, Mycotaxon 109: 502. 2009.

≡ *Rhizophagus custos* (C. Cano & Dalpé) C. Walker & A. Schüssler, The *Glomeromycota*: 19. 2010.

Specimen examined: Ex type specimens (Cano et al. 2009) isolated from cultures maintained at EEZ, CSIC in Granada, Spain (collection date 15.9.2013 by J. Palenzuela; ZT Myc 49021; examined by Oehl).

Rhizoglomus fasciculatum (Thaxt.) Sieverd., G.A. Silva & Oehl **comb. nov.**

MycoBank MB 803197

≡ *Endogone fasciculata* Thaxt., Proc. Am. Acad. Arts & Sci. 57: 308. 1922.

≡ *Glomus fasciculatum* (Thaxt.) Gerd. & Trappe, Mycol. Mem. 5: 51. 1974.

≡ *Rhizophagus fasciculatus* (Thaxt.) C. Walker & A. Schüssler, The *Glomeromycota*: 19. 2010.

= *Endogone arenacea* Thaxt., Proc. Am. Acad. Arts & Sci. 57: 317. 1922

= *Rhizophagites butleri* Rosend., Bull. Torrey Bot. Club 70: 131. 1943.

Specimen examined: Non-type specimen (morphology following Thaxter 1922; sensu Walker & Koske 1987) collected in bait cultures from Switzerland, near Basel (collection date 1.10.2005); examined by Oehl (ZT Myc 30478).

Rhizoglomus intraradices (N.C. Schenck & G.S. Sm.) Sieverd., G.A. Silva & Oehl **comb. nov.**

MycoBank MB 803192

≡ *Glomus intraradices* N.C. Schenck & G.S. Sm., Mycologia 74: 78. 1982.

≡ *Rhizophagus intraradices* (N.C. Schenck & G.S. Sm.) C. Walker & A. Schüssler, The *Glomeromycota*: 19. 2010.

Specimen examined: Type described from a pot culture by Schenck & Smith (1982). Isolated from Florida State at the USDA Horticultural Research Station in Orlando. Type OSC #40255, examined by Oehl at OSC in 2002. Non-type from a pot culture (SAF51) established by Oehl, isolated from a temporary *Lolium perenne* grassland in Biengen (Germany), near Freiburg im Breisgau (ZT Myc 30479); examined by Oehl.

Rhizoglomus invermaium (I.R. Hall) Sieverd., G.A. Silva & Oehl **comb. nov.**

MycoBank MB 808609

≡ *Glomus invermaium* I.R. Hall, Trans. Br. Mycol. Soc. 68: 345. 1977.

Specimen examined: Isotypes after Hall (1977) deposited at OSC #43941. Hall & Abbott (1979) photographic slide collection representing the type specimens deposited at PDD herbarium; examined by Oehl. Several greenhouse pot cultures of *Gl. invermaium*, established from single spores on host plant *Hieracium pilosella* (so-called monosporic cultures) at SAF (see http://www.agroscope.admin.ch/bodenoekologie/08050/08067/08068/index.html?lang=en; accessions SAF205-208) were examined. Specimens from three cultures were deposited at Z+ZT (ZT Myc 57170-57172), and first sequences from these cultures at EMBL-EBI (LN624111-LN6241112; Säle et al., unpublished).

Rhizoglomus irregulare (Błaszk., Wubet, Renker & Buscot) Sieverd., G.A. Silva & Oehl **comb. nov.**

MycoBank MB 803198

≡ *Glomus irregulare* Błaszk., Wubet, Renker & Buscot, Mycotaxon 106: 252. 2009.

≡ *Rhizophagus irregularis* (Błaszk., Wubet, Renker & Buscot) C. Walker & A. Schüssler, The *Glomeromycota*: 19. 2010.

Specimen examined: Type described from a pot culture by Błaszkowski et al. (2009). Isolated from Poland near Szczecin. Type (DPP, nowadays DEPE). Type specimen examined by Oehl at SAF from a type culture maintained in Basel in 2008 (ZT Myc 30480). Non-types from pot cultures established by Oehl. Isolated from three Swiss long-term experiments in Therwil near Basel, in Frick and in Zollikofen near Bern, respectively (ZT Myc 30481; ZT Myc 30482 and ZT Myc 30483); examined by Oehl in 2008-2014.

Rhizoglomus manihotis (R.H. Howeler, Sieverd. & N.C. Schenck) Sieverd., G.A. Silva & Oehl **comb. nov.**

MycoBank MB 803199

≡ *Glomus manihotis* R.H. Howeler, Sieverd. & N.C. Schenck, Mycologia 76: 695. 1984.

≡ *Rhizophagus manihotis* (R.H. Howeler, Sieverd. & N.C. Schenck) C. Walker & A. Schüssler, The *Glomeromycota*: 19. 2010.

Specimen examined: Type described from a pot culture on *Pueraria phaseoloides* (Roxb.) Benth. by Schenck et al. (1984). Isolated from Colombia near Quilichao (Cauca). Type OSC #41495, #41498, specimen examined by Oehl at OSC in 2002, and by Sieverding who cultivated the type under the accession C-1-1 at CIAT (ZT Myc 30484).

Rhizoglomus microaggregatum (Koske, Gemma & P.D. Olexia) Sieverd., G.A. Silva & Oehl **comb. nov.**

MycoBank MB 803200

≡ *Glomus microaggregatum* Koske, Gemma & P.D. Olexia, Mycotaxon 26: 125. 1986.

Specimen examined: Holotype described from soil by Koske et al. (1986) from USA, Michigan, Benzie Co. Holotype OSC #46719; examined by Oehl at OSC in 2002.

Rhizoglomus natalense (Błaszk., Chwat & B.T. Goto) Sieverd., G.A. Silva & Oehl **comb. nov.**

MycoBank MB 810477

≡ *Rhizophagus natalensis* Błaszk., Chwat & B.T. Goto, Mycotaxon 129: 100. 2014.

Specimen examined: Type described from pot-cultured *Plantago lanceolata* by Błaszkowski et al. (2014). Isolated from a sand dune in Parque Estadual das Dunas de Natal "Journalista Luiz Maria Alves" (Rio Grande do Norte, Brazil); examined by Oehl in 2014. One slide deposited at URM (URM 87580).

Rhizoglomus proliferum (Dalpé & Declerck) Sieverd., G.A. Silva & Oehl **comb. nov.**

MycoBank MB 803201

≡ *Glomus proliferum* Dalpé & Declerck, Mycologia 92: 1180. 2000.

≡ *Rhizophagus proliferus* (Dalpé & Declerck) C. Walker & A. Schüssler, The *Glomeromycota*: 19. 2010.

Specimen examined: Type described from monoxenic culture by Declerck et al. (2000) from Neufchateau, Guadeloupe, France. Isotype MUCL 41827, examined by Oehl from a pot culture maintained in Basel in 2008 (ZT Myc 30485).

Some species that superficially resemble *Rhizoglomus* species (e.g., *G. diaphanum*, *G. pallidum*, and *G. vesiculiferum*) were not considered, as their spore formation might differ from that of *Rhizoglomus* and their molecular phylogeny has not yet been fully determined or remains unclear.

Discussion

The name of the new genus, *Rhizoglomus,* is based on the ability of its species to form abundant spores, spore clusters, or sporocarps in the bulk soil and soil rhizosphere and additionally within roots. Formation of spores within roots of host plants is, however, not unique to *Rhizoglomus*. Certain species of other, phylogenetically distant, genera also form spores inside roots, e.g., *Claroideoglomus lamellosum* (Dalpé et al. 1992, as *Glomus lamellosum*), *Septoglomus xanthium* (Błaszkowski et al. 2004, as *G. xanthium*), *Archaeospora myriocarpa* (Schenck et al. 1986, as *Acaulospora myriocarpa*), and *Entrophospora infrequens* (Sieverding & Toro 1985, Sieverding & Oehl 2006).

Rhizoglomus species, however, differ from other *Glomeraceae* (sensu Oehl et al. 2011a,b) in that they possess a pore or channel that is usually open at the connection between spore and the cylindric subtending hypha. One or a few septa may form in the hyphae at some distance from the spore base. Mature spores of most *Rhizoglomus* spp. have two or up to five distinct layers of which one to three hyaline to subhyaline outer layers may separate from the inner, persistent (= structural), and usually pigmented layer(s) when spores are squeezed. In some species, such as *R. intraradices* and *R. irregulare,* the

outer hyaline to subhyaline layers may degrade or slough away from the inner persistent layer(s).

In spores of *Glomus* spp., the basal channel or pore is generally obstructed by wall material or proper septa (Oehl et al. 2011b). In *Funneliformis*, the hyphal attachment is characteristically funnel-shaped (Oehl et al. 2011b). *Simiglomus* resembles *Rhizoglomus* in its similarly open-pored hyphal attachment, but its hyphal walls are substantially thicker and thicken over longer distances than in *Rhizoglomus*. *Septoglomus* spp. have regular septa or strong plugs at the spore base (Oehl et al. 2011b). The taxonomic separation of *Rhizoglomus* based on morphological characteristics was corroborated by molecular studies that placed *Rhizoglomus* spp. in a separate clade (Schüßler & Walker 2010, Krüger et al. 2012); this clade was formerly called the „*Glomus* group Ab2" (Oehl et al. 2011b).

To date, no *Rhizoglomus* species has been reported to cause a root disease resulting in the dieback or death of a plant. *Rhizoglomus* comprises 'key species' of AM fungi with regard to scientific and economic importance, such as *R. intraradices* and *R. irregulare* (e.g., Stockinger et al. 2010). The latter, formerly often confused with *R. intraradices* (Stockinger et al. 2010), develops well on axenic root cultures (Chabot et al. 1992) and has therefore been used as a model organism for AM fungal genetics (e.g., Jansa et al. 2002, Börstler et al. 2008, Croll et al. 2008, 2009). These AM fungi are also key components of many of the commercially available AM fungal products. Further from our extensive re-examination, both morphologically and molecularly, of the various specimens and cultures and our re-evaluation of the literature, we are convinced that such important AM fungal species should be placed correctly in *Glomeromycota* and not erroneously assigned to a pathogenic genus, such as *Rhizophagus*.

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First report of the pantropical species *Diploschistes rampoddensis* from Europe

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Abstract — The lichen species *Diploschistes rampoddensis*, previously known only from a few localities in tropical and subtropical Asia and Oceania, is reported here for the first time in Europe. A detailed description, including macro- and microscopic characters, and comparisons with closely related taxa are also provided. Molecular analyses based on the nrITS were used to confirm this new record for the European lichen biota.

Key words — *Ascomycota,* BAli-phy, disjunct distribution, lichenized-fungi, *Ostropales*

Introduction

The lichen genus *Diploschistes* Norman (*Graphidaceae*, *Ostropales*, *Ascomycota*) currently includes 45 species (Kirk et al. 2008, Pérez-Vargas et al. 2012, Fernández-Brime et al. 2013), distributed mainly in temperate and arid regions of both hemispheres, with only a few species restricted to the tropics (Lumbsch 1989). One of the few species occurring in tropical areas is *D. rampoddensis,* a taxon that was first described by Nylander (1900, as *Urceolaria rampoddensis*) from the locality of Rampodde (Sri Lanka). According to the literature (Nylander 1900, Lumbsch 1993, Pant & Upreti 1993, Martín et al. 2003) and unpublished herbarium specimens, this species appears to be quite rare and has previously been recorded only from a few localities in tropical and subtropical Asia and Oceania.

As part of an ongoing project to assess the lichen diversity of several protected areas of Catalonia (NE Spain), we intensively surveyed the cliffs of Sant Llorenç

de la Muga, a protected area included in the Plan of Sites of Natural Interest (PEIN). Among several *Diploschistes* collections, three samples were initially identified as *D. rampoddensis*, based on their morphological, anatomical, and chemical features. As these specimens were, however, rather scant or inconspicuous, we checked the identification using the nuclear ribosomal internal transcribed spacer (nrITS), recently selected as the universal DNA barcode marker for fungi (Schoch et al. 2012). Our molecular analyses further confirmed that these specimens represent *D. rampoddensis,* which extends its distributional range to Europe.

Materials & methods

Morphological analysis

The morphological study was carried out on fresh specimens collected from the cliffs of Sant Llorenç de la Muga and on herbarium material of *Diploschistes rampoddensis* lodged and loaned from UPS, as well as on other taxa of *Diploschistes* used for comparison. Morphological characters were examined with an Olympus VMZ dissecting microscope (40×). Anatomical characters were studied on hand-cut sections mounted in water (40×) or oil immersion (100×) using an Olympus CH-2 light microscope. Vouchers of new specimens were conserved in CeDocBiV , Universitat de Barcelona, Spain (BCN), Institute of Botany, Academy of Sciences, Czech Republic (PRA), and the first author's private herbarium (Hb. Fdez.-Brime, SFB).

Selected specimens were photographed using a Pixera Pro150 ES camera mounted on an Olympus SZ60 stereomicroscope. For each selected specimen, multiple images in different focal planes were taken and subsequently assembled into a final unique picture using the software Helicon Focus 5.2.

Spot tests were performed using standard lichenological chemical reagents (K, C) along with thin-layer chromatography (TLC) following the protocols of Orange et al. (2001).

Molecular analysis

Genomic DNA was obtained from small pieces of thallus and apothecia sampled from fresh specimens using a phenol-chloroform-isoamyl alcohol extraction protocol based on Lee et al. (1988). Approximately 560 bp including the complete nrITS region was amplified using the primers ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990). PCR mixtures were prepared as in Gueidan et al. (2007) and conditions for thermocycling followed Fernández-Brime et al. (2013). The nrITS region was sequenced as detailed in Martín & Winka (2000), using the same amplification primers.

We aligned the nrITS of the three specimens tentatively identified as *Diploschistes rampoddensis* with the only nrITS sequence of *D. rampoddensis* available from GenBank, together with other sequences of closely related representatives of *Diploschistes* subg. *Diploschistes* (Fernández-Brime et al. 2013). *Diploschistes actinostomus* (Ach.) Zahlbr. and *D. candidissimus* (Kremp.) Zahlbr., from *Diploschistes* subg. *Limborina* Fdez.-Brime et al. (Fernández-Brime et al. 2013) were selected as outgroups to root the phylogeny. A total of 17 sequences were used for the phylogenetic analyses (Table 1). All newly

Table 1. Specimens and sequences of *Diploschistes* used in the present phylogenetic analysis.

Species	Voucher	Origin	GenBank*
D. actinostomus	BCC-Lich 13394	Spain, Catalonia	AF229194
D. candidissimus	ESS 20699	Australia, South Australia	AJ458281
D. cinereocaesius	ESS 9364	Venezuela, Mérida	AJ458282
	DUKE 0047509	Costa Rica, San José	HQ650715
	Hb. Palice, Palice 4471	Ecuador	**KJ542542**
D. rampoddensis	Hb. Lumbsch, Aptroot 39679	Papua New Guinea	AJ458286
	BCC-Lich 18009	Spain, Catalonia	KC166992
	BCC-Lich 18011	Spain, Catalonia	KC166993
	BCC-Lich 18008	Spain, Catalonia	**KJ542543**
D. scruposus	BCN-Lich 19326	Spain, Catalonia	KC167067
	Hb. Fdez.-Brime, SFB 59	Spain, Catalonia	**KJ542545**
	Hb. Fdez.-Brime, SFB 103	Spain, Catalonia	**KJ542546**
	ESS 21508	Germany, Rheinland-Pfalz	AJ458287
D. scruposus "*interpediens*"	BCN-Lich 19319	Spain, Catalonia	KC166995
	BCN-Lich 19355	Spain, Catalonia	KC166999
	BCN-Lich 19322	France, Languedoc-Roussillon	KC167003
	Hb. Fdez.-Brime, SFB 104	Spain, Catalonia	**KJ542544**

* Newly obtained sequences shown in bold.

generated DNA sequences are now deposited in GenBank (KJ542542–KJ542546) and the alignment is available in TreeBASE (http://www.treebase.org; ID number 15496).

Sequences were manually aligned with Mesquite 2.75 (Maddison & Maddison 2011). Terminal primers and partial nuSSU and nuLSU were excluded from the alignment. Phylogenetic relationships were inferred using weighted maximum parsimony (wMP) and Bayesian methods (B). The wMP analyses were carried out in PAUP* v.4.0b10 (Swofford 2002), with gaps treated as a fifth character state in unambiguously aligned regions. These regions were subjected to symmetric step matrices (i.e., ITS1, 5.8S, and ITS2 being treated separately), using STMatrix v.3.0 (F. Lutzoni & S. Zoller, Dept. of Biology, Duke University), as outlined in Gaya et al. (2003). Ambiguously aligned regions were removed from the wMP searches and recoded and incorporated into the analyses using INAASE v.2.3b (Lutzoni et al. 2000), as in Gaya et al. (2008). All wMP analyses were performed using heuristic searches with 1000 random addition sequences (RAS), the tree bisection-reconstruction (TBR) algorithm, MULTREES in effect, and collapsing branches with maximum branch length equal to zero. Branch support (BS) was assessed with 1000 bootstrap replicates (Felsenstein 1985) with full heuristic searches, and 2 RAS per bootstrap replicate. In all BS analyses, the same parameters as in the original MP search were used, and constant sites were excluded from all analyses. The Bayesian analyses (B) were done using BAli-Phy 2.1.1 (Suchard & Redelings 2006), a software that simultaneously accounts for alignment uncertainty and phylogeny, allowing the inclusion of the whole alignment. In this case, the nrITS dataset was divided into three partitions (ITS1, 5.8S, and ITS2) as suggested in Gaya et

al. (2011). The analyses were run under the following settings: ITS1 and ITS2 sharing the same model of evolution (HKY+G), indel model (RS07; Redelings & Suchard 2007) and branch length estimation, while the EQU model and separate branch length estimation were applied to the partition 5.8S, which was fixed because the alignment was unambiguous. We ran 6 independent Markov chains of 50,000 iterations each, sampling the Markov chains every 10 iterations. After discarding the first 1250 samples of each run (25%), we computed the majority rule consensus with the remaining trees (22,500).

Results & discussion

Molecular phylogenetics

The final nrITS alignment included 17 taxa and comprised 594 characters. We excluded from the wMP analyses 557 characters, of which 407 were constant sites and 150 corresponded to 19 ambiguously aligned regions. The characters from the ambiguously aligned regions were included as 19 INAASE recoded characters. From the 56 variable characters analyzed by wMP, 40 were parsimony-informative. We included the whole alignment for the B analyses.

The wMP search yielded six most parsimonious trees of 159.27 steps, which were found in one island hit 991 times out of 1000 RAS. The strict consensus tree (Fig. 1) shows 13 resolved internodes, of which nine were significantly supported (BS ≥70%). The 50% majority rule consensus tree from the B analyses showed slightly less resolution, with nine resolved internodes, but, as with wMP, nine of these were significantly supported (PP ≥0.95).

The three European specimens putatively representing *Diploschistes rampoddensis* clustered with the only sequence of *D. rampoddensis* from GenBank and formed a well-supported clade (BS = 100%; PP = 1). This clade was sister to *D. cinereocaesius* (Sw.) Vain., which provided support for this sister relationship for the first time. In contrast, specimens of other taxa [e.g., *Diploschistes scruposus* (Schreb.) Norman, *D. scruposus* "morphotypus *interpediens*")], even if morphologically similar and collected from the same locality of Sant Llorenç de la Muga (specimens marked with an asterisk in Fig. 1), clustered separately from *D. rampoddensis*.

Taxonomy

Diploschistes rampoddensis (Nyl.) Zahlbr., Cat. Lich. Univers. 2: 665, 1924. Fig. 2A

Thallus saxicolous, crustose, yellowish grey to greenish yellow, without pruina but with a crystalliferous surface, 0.2–0.9 µm thick, friable (easily broken), from verrucose-areolate to mostly reduced into conglutinated granules; epinecral layer 24–31 µm thick, without a well-developed cortex; photobiont trebouxioid.

Apothecia urceolate, 0.2–1.3(–1.7) mm in diam., never subdivided, with a deeply concave black disc with white pruina. Excipulum black, 60–100

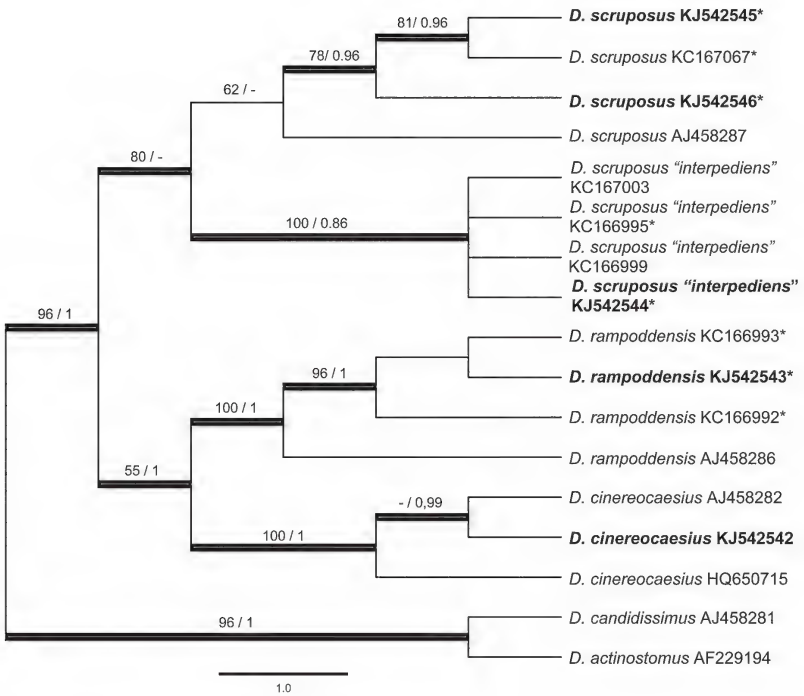

FIG. 1. Phylogenetic relationships of sequences of *Diploschistes* species summarized by a strict consensus tree of six equally most parsimonious trees, as revealed by the wMP analyses of the nrITS dataset, including 19 INAASE recoded characters. Bootstrap support ≥50% (BP; left) and posterior probabilities ≥0.5 (PP; right) are shown above branches. Internodes with BP values ≥70% or PP ≥0.95 are highlighted by thicker lines. Asterisk-marked specimens collected in the same locality of Sant Llorenç de la Muga (Catalonia, Spain). Newly generated sequences are presented in bold font.

(–130) µm thick, lateral paraphyses present. HYMENIUM hyaline to pale brown, (78–)98–128(–134) µm high. ASCI clavate, unitunicate, non-amyloid walls, (6–)8 spores per ascus, 100–170 × 15–30 µm. ASCOSPORES ellipsoid, pale to dark brown, non-amyloid, (19.5–)21– 26.5(–29) × (8.5–)9–12(–13.5) µm (n = 59), with 4–7(–8) transverse septa, and 1–2 longitudinal septa.

CHEMISTRY — Spot test reactions of the thallus K– or K+ pale yellow, C+ and KC+ pink (brief reaction). TLC: lecanoric acid as major compound, compounds in traces were not found.

DISTRIBUTION & HABITAT — Since *Diploschistes rampoddensis* was first described by Nylander (1900) from material collected in Rampodde, Sri

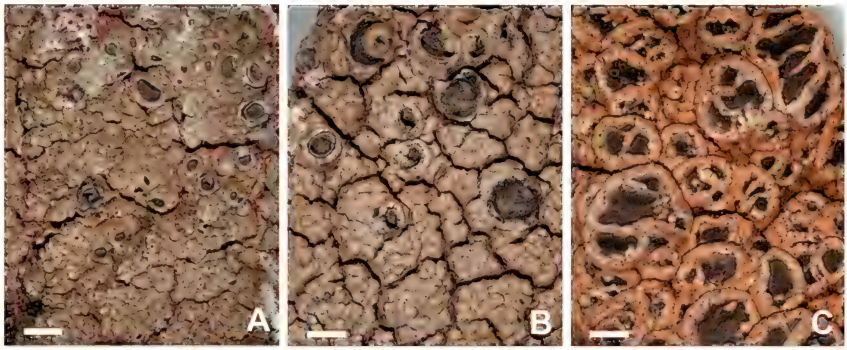

Fig. 2. A. *Diploschistes rampoddensis* (BCN-lich. 18011): habit. B. *D. scruposus* "*interpediens*" (BCN-lich. 14404): habit. C. *D. cinereocaesius* (BCN-lich. 18010): habit. Scale bars = 1 mm.

Lanka, it has been recorded from a few Asian and Australasian localities: China (see examined specimens), India and Nepal (Pant & Upreti 1993), Indonesia (Lumbsch 1993), and Papua New Guinea (Martín et al. 2003). The lichen usually occurs in lowlands and only rarely appears at high elevations of the intertropical region (Lumbsch 1993). Our study significantly extends its distribution to Europe, where it is reported only from a single locality, Sant Llorenç de la Muga (Catalonia, Spain). This area's warm humid Mediterranean climate (with relatively high temperatures and humidity) has allowed the establishment of a rich foliicolous lichen flora (Llop & Gómez-Bolea 2006), predominantly Atlantic bryophyte species such as *Leucobryum juniperoideum* (Brid.) C. Müll. (Brugués et al. 1974), and the pteridophyte *Pellaea calomelanos* (Sw.) Link earlier known from South Africa, northern India, and Macaronesia (Terradas & Brugués 1973). The existence of these Atlantic and paleotropical elements in the study area would agree with the presence of *Diploschistes rampoddensis* outside its known pantropical distribution.

The European *D. rampoddensis* samples are saxicolous and grow on Garumnian red beds (HCl–). Pant & Upreti (1993), however, reported the species colonizing sandstones from India.

Specimens examined — **CHINA. Yunnan.** Simao Distr. Mojiang Co., Mojiang (km-post 350 from Kunming), along the main road, 23°26′N 101°41′E, 1200 m, on hard soil, 12 Sep. 1987, Moberg & Santesson 7702 (UPS L-11539); along the road E of the river Bavian Jiang (km-post 433 from Kunming), 23°16′N 101°16′E, 1100 m, on wayside rocks, 13 Sep. 1987, Moberg & Santesson 31970 (UPS L-11540). **SPAIN. Catalonia.** Sant Llorenç de la Muga, path to Can Gener, UTM 31TDG8388, 150 m, Garumnian red beds, 9 Feb. 2007, Llimona & Hladun (BCN-lich. 18011); cliffs by the river la Muga, near an old mine, UTM 31TDG8188, 280 m, Garumnian red beds 45° S-SW, 2 Mar. 2007, Llimona, Hladun & Muñiz (BCN-lich. 18008, GenBank KJ542543; BCN-lich. 18009).

Remarks —*Diploschistes rampoddensis* resembles octosporate specimens of *D. scruposus* (Fig. 2B; *D. scruposus* "morphotypus *interpediens*", hereafter abbreviated to *D. scruposus* "*interpediens*"), which also have (6–)8 ascospores per ascus and a similar ascospore size [(21–)23.5–27 × (9.5–)10–13(–13.5) µm]. However, a detailed morphological comparison reveals that *D. rampoddensis* has a thinner and more delicate thallus than *D. scruposus* "*interpediens.*" Their chemistry also differs: *D. rampoddensis* contains only lecanoric acid (major), while *D. scruposus* "*interpediens*" contains diploschistesic acid (minor) and orsellinic acid (minor), in addition to lecanoric acid (major) (Barbero 1998). Morphological and chemical differences between these two taxa are nonetheless really subtle, and the utilization of the nrITS was crucial in confirming the identity of the European specimens of *D. rampoddensis.*

Within *Diploschistes,* there are two other pantropically distributed species (*D. cinereocaesius* and *D. hypoleucus* Zahlbr.), which resemble *D. rampoddensis* in having a yellowish thallus, octosporous asci, and a rather similar ascospore size. These two species, however, are terricolous, have bigger apothecia that are usually secondarily subdivided (see *D. cinereocaesius,* Fig. 2C), and have different chemistry: *D. cinereocaesius* contains lecanoric acid (major) and diploschistesic acid (minor), while *D. hypoleucus* contains gyrophoric acid as a major compound. That *Diploschistes rampoddensis* and *D. cinereocaesius* appear as sister taxa in our phylogeny suggests a biogeographic pattern.

Additional specimens examined — *Diploschistes cinereocaesius.* **COLOMBIA. Urrao.** Páramo de Frontino, 6°25′N 76°05′W, 3500 m, 30 June 1985, Churchill, Sastre-De Jesús & Escobar (DUKE 0144448). **ECUADOR. Imbabura.** Laguna Cuicocha - NE edge, ca 9km W of town Cotacachi, 00°18′43″ N, 78°21′15″ W, on soil at trail-cutting, 3300–3350 m, 8 Oct. 2000, Palice 4471 & Soldán (PRA, GenBank KJ542542) **PERU. Huaraz.** Route to Laguna Churup, 9°29′S 77°26′W, 4070 m, 25 Aug. 2010, Rothfels 4000 & Zylinski (BCN-lich. 18010).

Diploschistes scruposus. **SPAIN. Catalonia.** Girona, Sant Llorenç de la Muga, cliffs by the river la Muga, near an old mine, UTM 31TDG8188, 280 m, Garumnian red beds 45° S-SW, 2 Mar. 2007, Llimona, Hladun & Muñiz (Hb. Fdez.-Brime, SFB 59, GenBank KJ542545; BCN-Lich 19326); cliffs by the river la Muga, UTM 31TDG8188, 240 m, granitic outcrops 40° S-SW, 3 June 2009, Fernández-Brime (Hb. Fdez.-Brime, SFB 103, GenBank KJ542546).

D. scruposus "*interpediens*". **FRANCE. Pyrenées Orientales:** Albera, road from Maurellans to Rinoguers, 31TDH85351, 300 m, granitic outcrops near the road, 13 June 2009, Llimona (BCN-Lich 19322). **SPAIN. Catalonia.** Barcelona, Òrrius, turó de Céllecs, 31T DG4400, 530 m, granitic outcrops, 23 Jan. 2009, Llimona & Fernández-Brime (BCN-Lich 19355). Girona, Sant Llorenç de la Muga, path to Can Gener, 31T DG8387, 140-150 m, Garumnian red beds, 9 Feb. 2007, Llimona & Hladun (BCN-lich. 19319); cliffs by the river la Muga, UTM 31TDG8188, 260 m, granitic outcrops 60° E, 3 June 2009, Fernández-Brime (Hb. Fdez.-Brime, SFB 104, GenBank KJ542544). **La Rioja.** Anguiano, electric power station, 30T WM1878, 670 m, *Quercus ilex* forest, 8 Sep. 2004, Hladun & Muñiz (BCN-Lich 14404).

Acknowledgments

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MYCOTAXON

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New species of *Graphium* and *Periconia* from China

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Abstract—Three new species from soil in China, *Graphium variabile, G. wuweiense,* and *Periconia guangdongensis,* are described and illustrated. Specimens (dried cultures) and living cultures are deposited in the Herbarium of Shandong Agricultural University, Plant Pathology (HSAUP), and the Herbarium of Institute of Microbiology, Academia Sinica (HMAS).

Key words — dematiaceous hyphomycetes, anamorphic fungi, taxonomy

Introduction

Graphium was established by Corda (1837) and is characterized by possession of synnematous conidiophores with each synnema capped by a slimy conidial head. The conidiogenous cells are monoblastic, percurrent, subulate or cylindrical. Conidia are produced from annellides, often aggregated in slimy heads, cylindrical rounded at the apex, cuneiform or ellipsoidal, usually with a flat base, and 0-septate. Although Index Fungorum (2014) lists 173 taxa (many infraspecific), Seifert et al. (2011) estimates that the genus may contain only 19 authentic species.

Periconia was established by Tode (1791) and is characterized by macronematous, mononematous conidiophores. The conidiogenous cells are monoblastic, polyblastic, branched, and ellipsoidal. Conidia are catenate, spherical or subspherical, verruculose or echinulate, 0-septate and aggregated in heads. Seifert et al. (2011) estimates that the genus may contain 40 authentic species.

During a survey of soil dematiaceous hyphomycetes in China, several unusual species of *Graphium* and *Periconia* were collected. Two *Graphium* and one *Periconia* species are described and illustrated as new.

^Y.M. Wu and J.J. Xu contributed equally to this work.

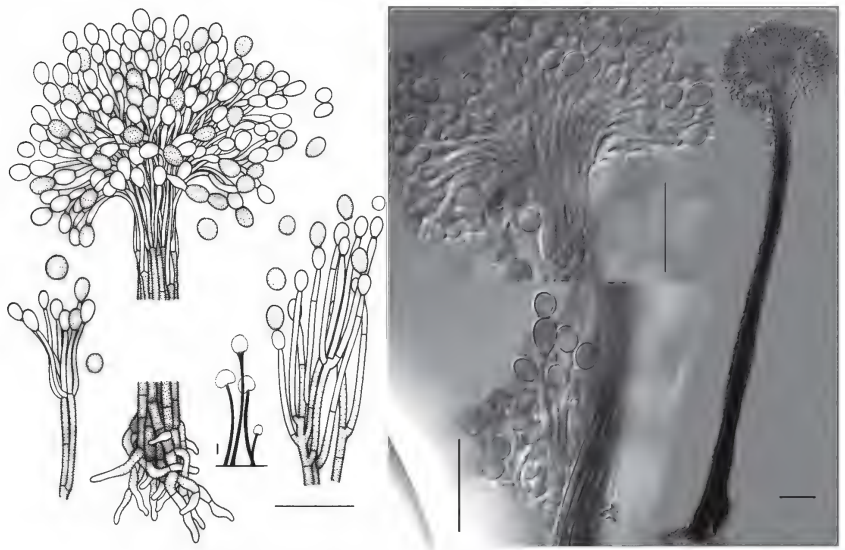

FIG. 1. *Graphium variabile* (ex holotype HSAUP $II_{04}2789$).
Conidia, conidiophores, conidiogenous cells, and synnemata. Scale bars = 25 μm.

Graphium variabile J.J. Xu & T.Y. Zhang, **sp. nov.** FIG. 1

MYCOBANK MB 807387

Differs from *Graphium eumorphum* by its conidia that are smaller and contain no oil droplets and from *G. terricola* by its larger conidia.

TYPE: China, Fujian Province, Xiamen National Forest Park, from a forest soil, 6 Oct. 2004, J.J. Xu (**Holotype** HSAUP $II_{04}2789$; **isotype** HMAS 196278).

ETYMOLOGY: The epithet refers to the variably shaped conidia.

COLONIES on CMA (Matsushima 1995) slow growing, effuse, felted, white at initial stage, then darkish brown to black. MYCELIUM partly superficial, partly immersed in the substratum, hyphae branched, septate, smooth, subhyaline, 2–4 μm wide. SYNNEMATA erect, scattered, rough, brown at base paler towards the apex, 150–320 μm long, 15–30 μm wide at base, slightly splayed out at the apex. CONIDIOGENOUS CELLS annellides, subhyaline, subulate or cylindrical, integrated, terminal, smooth, 15–25 × 1.5–2.5 μm. CONIDIA acrogenous, subhyaline to hyaline, forming conidial chains and enveloped in mucilaginous secretion, ellipsoidal, pyriform, obovoid to globose, smooth, 0-septate, globose conidia 3–6.5 μm in diameter, others 5.5–7 × 3.75–5 μm, wide in the broadest part, slightly truncate at the ends.

COMMENTS: Morphologically, *G. variabile* resembles *G. eumorphum* (Sacc.) Sacc. (Saccardo 1886) and *G. terricola* Manohar. et al. (Manoharachary et al. 1975). *Graphium eumorphum* is distinguished by longer conidia (6–8 × 3.5–4.5

μm, 2-guttulate) that contain oil droplets, while *G. terricola* produces smaller conidia (4–6.5 × 2–3.2 μm).

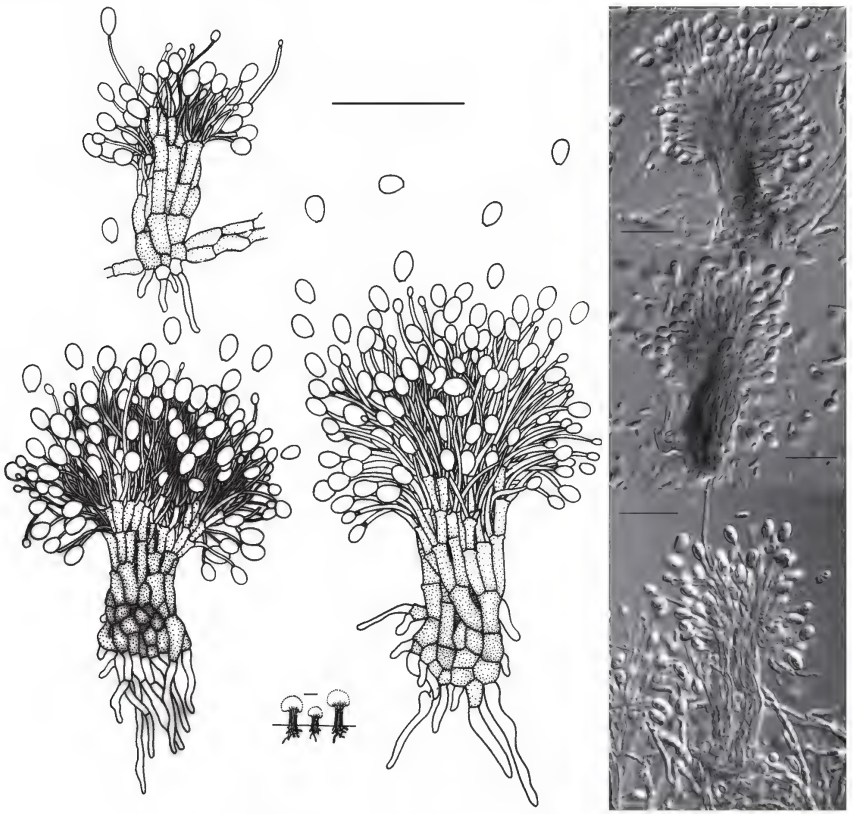

Fig. 2. *Graphium wuweiense* (ex holotype HSAUP II$_{05}$2647).
Conidia, conidiophores, conidiogenous cells, and synnemata. Scale bars = 25 μm.

Graphium wuweiense J.H. Kong & T.Y. Zhang, **sp. nov.** Fig. 2

MycoBank MB 807385

Differs from *Graphium adansoniae* and *G. penicillioides* by its shorter conidiophores.

Type: China, Gansu Province, Wuwei, from a desert oasis soil, 18 Apr. 2005, J.H. Kong (**Holotype** HSAUP II$_{05}$2647; **isotype** HMAS 196279).

Etymology: in reference to the type locality.

Colonies on CMA (Matsushima 1995) slow growing, effuse, white at initial stage, then darkish brown, felted. Mycelium mostly immersed in the substratum, hyphae branched, septate, smooth, subhyaline, 1.5–3 μm wide. Synnemata erect, scattered, smooth, brown at base, paler towards the apex,

20–40 μm long, 10–20 μm wide at base, slightly splayed out at the apex. Conidiogenous cells annellides, hyaline, subulate or cylindrical, integrated, terminal, smooth, 7–18 × 1.5–2.5 μm. Conidia acrogenous, hyaline, forming conidial chains enveloped in mucilaginous secretion, ellipsoidal, pyriform to obovoid, smooth, 0-septate, truncate at the base, 4–6 μm long, 3–5 μm wide.

Comments: Morphologically, *Graphium wuweiense* is most similar to *G. adansoniae* Cruywagen et al. (Cruywagen et al. 2010) and *G. penicillioides* Corda (Corda 1837). However, both of these species have much longer conidiophores (*G. adansoniae*, 88–150 μm; *G. penicillioides*, up to 200 μm).

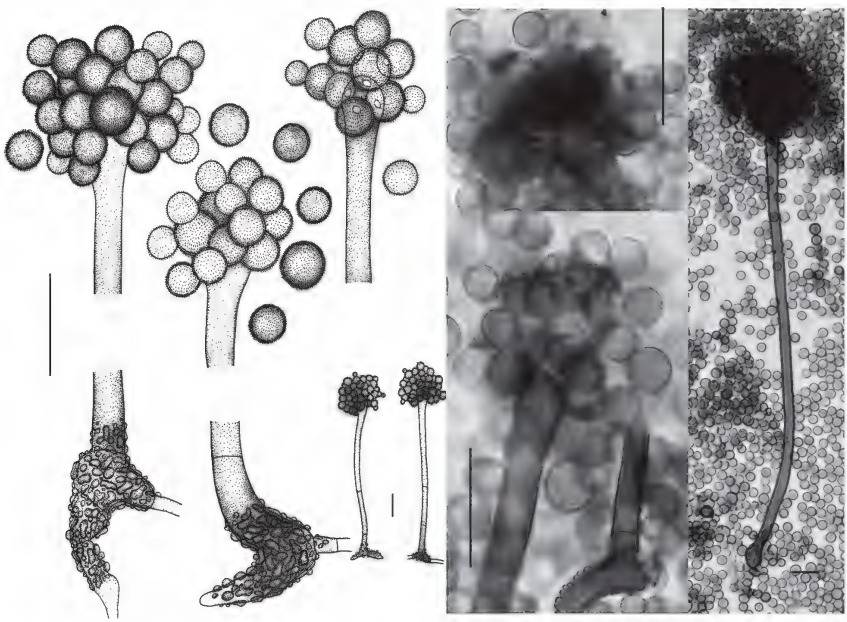

Fig. 3. *Periconia guangdongensis* (ex holotype HSAUP II $_{04}$3133). Conidia, conidiophores, and conidiogenous cells. Scale bars = 25 μm.

Periconia guangdongensis J.J. Xu & T.Y. Zhang, **sp. nov.** Fig. 3

MycoBank MB 807386

Differs from *Periconia cookei* and *P. pseudobyssoides* by its shorter globose conidiophores and smaller conidia.

Type: China, Guangdong Province, Meizhou, from a grassland soil, 10 Aug. 2004, J.J. Xu (**Holotype** HSAUP II $_{04}$3133; **isotype** HMAS 196280).

Etymology: in reference to the type locality.

Colonies on MEA (Lefebvre et al. 1949) effuse, initially white, then grey to greyish brown, cottony or fine hairy. Mycelium superficial or immersed,

hyphae branched, septate, sometimes rough, subhyaline, 1.5–4 µm wide. Conidiophores macronematous, mononematous, straight or slightly flexuous, smooth although often verrucose at base, pale brown to mid-brown, unbranched, 3–4-septate, 230–480 µm long, 7–9 µm wide at base, often swollen at apex. Conidiogenous cells globose, directly arising from the swollen apex, pale brown to dark brown, 5–9 µm diam. Conidia globose to subglobose, 0-septate, smooth initially but echinulate at maturity, brown to dark brown, 7–11 µm diam.

Comments: Morphologically, *Periconia guangdongensis* slightly resembles *P. cookei* E.W. Mason & M.B. Ellis (Mason & Ellis 1953) and *P. pseudobyssoides* Markovsk. & Kačergius (Markovskaja & Kačergius 2014). However, *P. cookei* has larger non-globose conidia (13 × 16 µm) and shorter conidiophores (88–150 µm), and *P. pseudobyssoides* has larger non-globose conidia (15 × 17 µm) with conidiophores up to 600 µm.

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Two new species of *Myrothecium* from the Qinghai-Tibet Plateau Area, China

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Abstract — Two new species from the Qinghai-Tibet Plateau Area (China), *Myrothecium variabile* and *M. xigazense,* are described and illustrated. Specimens (dried cultures) and living cultures are deposited in the Herbarium of Shandong Agricultural University, Plant Pathology (HSAUP), and the Herbarium of Institute of Microbiology, Academia Sinica (HMAS).

Key words — *Ascomycota,* dematiaceous hyphomycetes, soil fungi, *Stachybotriaceae,* taxonomy

Introduction

During a survey of dematiaceous soil hyphomycetes in China, several unusual *Myrothecium* specimens were collected, of which two represent new species. They are described and illustrated from cultures grown on potato dextrose agar (PDA) (Domsch et al. 2007).

Myrothecium Tode [type species: *M. inundatum* Tode (Tode 1790)] is characterized by conspicuous sporodochia that are cupulate, pustulate or stalked, synnema-like, formed of densely compacted conidiophores arising from a developed stroma and bearing a mass of slimy, green to black conidia. Sporodochia are surrounded by differentiated, often curling, marginal hairs. Conidiophores are hyaline or slightly olivaceous, and branch repeatedly to the ultimate branches, the phialides. Phialides are compacted in a dense parallel layer, cylindrical with conically tapering tips and usually undifferentiated collarettes. Conidia are 1-celled, subhyaline to pale greenish brown but dark olivaceous in mass. Seifert et al. (2011) listed 30 taxa for the genus.

^Yue-Ming Wu and Yu-Lan Jiang contributed equally to this work.

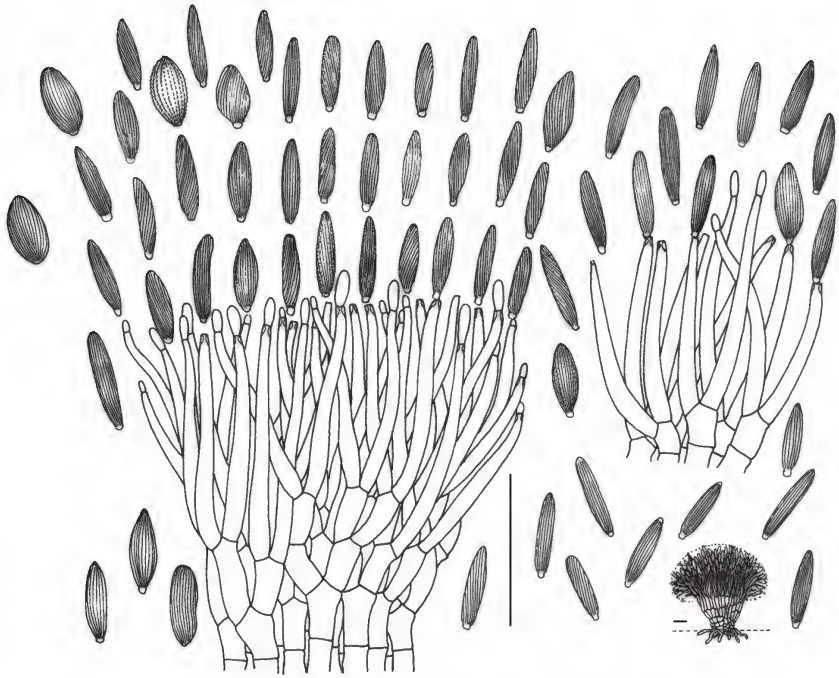

Fig. 1. *Myrothecium variabile* (ex holotype HSAUP II $_{07}$1083).
Conidia, conidiophores, and conidiogenous cells. Scale bars = 25 μm.

Myrothecium variabile Y.M. Wu & T.Y. Zhang, **sp. nov.** Fig. 1

MycoBank MB 808618

Differs from *Myrothecium cinctum* and *M. striatisporum* by its longer conidia.

Type: China, Tibet: Xigaze, altitude 3100 m, from a forest soil, 11 Sept. 2007, Y.M. Wu (**Holotype** HSAUP II $_{07}$1083; **isotype** HMAS 196284).

Etymology: The epithet refers to the variable shape of the conidia.

Colonies on PDA effuse, flocculent, pale yellow at initial stage, later green to black. Mycelium superficial or immersed, hyphae branched, septate, smooth, hyaline, 2–3 μm wide. Sporodochia usually not more than 1.0 mm diameter, often confluent, at first green, later black with a white margin, without setae. Conidiophores straight or flexuous, branched, smooth, with apical branches arranged penicillately. Conidiogenous cells phialides colourless, smooth, 10–25 × 2–2.5 μm. Conidia unicellular, cylindrical, clavate, fusiform to navicular (L/W=6–11.25), often slightly protuberant and truncate at the base, smooth, pale greenish brown, in mass greenish brown to black, with distinct longitudinal or oblique striations, 12.5–16.25 × 2.5–3.5 μm.

Comments: Morphologically, *Myrothecium variabile* resembles *M. cinctum* (Corda) Sacc. (Saccardo 1886) and *M. striatisporum* N.C. Preston (Preston 1948) its longitudinally or obliquely striate conidia. However, *M. cinctum* (6.5–14 × 2.5–4.5 μm) and *M. striatisporum* (7–12 × 2.5–3.5 μm) have smaller conidia and verrucose conidiophores.

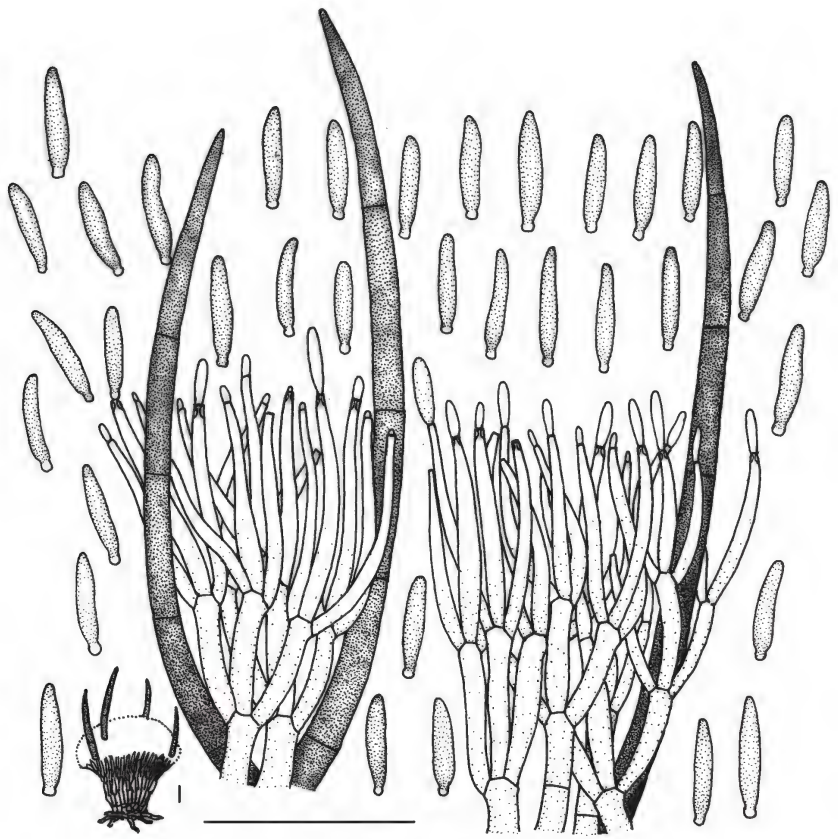

Fig. 2. *Myrothecium xigazense* (ex holotype HSAUP II $_{07}$0969).
Conidia, conidiophores, setae, and conidiogenous cells. Scale bars = 25 μm.

Myrothecium xigazense Y.M. Wu & T.Y. Zhang, **sp. nov.** Fig. 2

MycoBank MB 808619

Differs from *Myrothecium carmichaelii* and *M. jollymanii* by its longer conidia.

Type: China, Tibet: Xigaze, altitude 3900 m, from a grassland soil, 9 Sept. 2007, Y.M. Wu (**Holotype** HSAUP II $_{07}$0969; **isotype** HMAS 196285).

Etymology: in reference to the type locality.

Colonies on PDA effuse, flocculent, white at initial stage, later black, Mycelium mostly superficial, hyphae branched, septate, smooth, hyaline, 2–3 μm wide. Sporodochia usually not more than 1.2 mm diameter, often confluent, at first green, later black with a white margin. Setae pale brown, smooth, septate, 220–250 μm long, 4–6 μm thick. Conidiophores straight or flexuous, branched, with apical branches arranged penicillately. Conidiogenous cells phialides colourless, smooth, 8–15×2–2.5 μm. Conidia unicellular, cylindrical or clavate, often slightly protuberant and truncate at the base, smooth, pale greenish brown, dark greenish in mass, 12.5–15 × 2–3 μm.

Comments: Morphologically, *Myrothecium xigazense* resembles *M. carmichaelii* Grev. (Greville 1825) and *M. jollymanii* N.C. Preston (Preston 1948) in conidial colour and shape. However, both *M. carmichaelii* (10–11 × 1–1.3 μm) and *M. jollymanii* (10–12 × 2–2.5 μm) have shorter conidia. Additionally, *M. carmichaelii* lacks setae while the setae in *M. jollymanii* are hyaline, not pale brown as in *M. xigazense*.

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Nomenclatural novelties in the *Postia caesia* complex

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Abstract – Within the genus *Postia*, the *P. caesia* complex forms a distinctive morphological group. Based on recent molecular data, the current taxonomic status of the *P. caesia* complex is discussed and the nomenclature of the related taxa is revised as well. New combinations are: *Postia* subg. *Cyanosporus*, *Postia africana*, *Postia amyloidea*, *Postia caesioflava*, and *Postia coeruleivirens*.

Keywords – polypores, basidiomycetes, *Oligoporus*

Introduction

Based on recent molecular phylogenetic studies, the cosmopolitan polypore genus *Postia* Fr. belongs to the antrodia clade, with members characterized by brown-rot wood decay (Hibbett & Donoghue 2001, Binder et al. 2013). Within the genus, the *Postia caesia* complex forms a distinctive morphological group (Ţura et al. 2008). The foremost species of this complex is *P. caesia* (Schrad.) P. Karst., which was described from Germany (Schrader 1794) but probably has a circumglobal distribution (e.g., Pildain & Rajchenberg 2012, Ryvarden & Gilbertson 1994). The main morphological features of *P. caesia* are annual soft white basidiocarps that become blue-grey when bruised or spontaneously in age and small cylindrical to allantoid cyanophilous basidiospores (Niemelä 2013). In addition to *P. caesia* are similar taxa described from Europe (David 1974, 1980; Niemelä et al. 2001; Pieri & Rivoire 2005) and worldwide (Corner 1989). Species delimitation in this difficult complex has not been sufficiently clarified (Yao et al. 2005), and the generic name of this peculiar polypore group has changed more than once, depending on the various taxonomic concepts held in the past. The current taxonomic status of the *P. caesia* complex is discussed based on recent molecular data and the nomenclature of the related taxa is revised accordingly.

Nomenclature and taxonomy of *Boletus caesius*

McGinty (1909) proposed a new monotypic genus (*Cyanosporus*) for *Polyporus caesius* (Schrad.) Fr., based on its cyanophilous spores. However, the name *Cyanosporus caesius* (Schrad.) McGinty was not accepted in significant studies (e.g., Donk 1960, Jahn 1963, Lowe 1975), while the combination introduced earlier by Murrill (1907), *Tyromyces caesius,* was commonly used. After it became more evident that the species in *Tyromyces* P. Karst. were white-rot-producing fungi, David (1980) placed the brown-rot taxa into *Spongiporus* Murrill, Jülich (1982) combined them into *Postia,* and Gilbertson & Ryvarden (1985) transferred them into *Oligoporus* Bref. Depending on the biological, morphological and anatomical features of the type species, these genera also have been regarded as one genus. *Postia* (Fries 1874) has priority over *Oligoporus* (Brefeld 1888) on the basis of wide acceptance of the validity of Fries's 1874 publication (Karsten 1879, 1881; Donk 1960, Jülich 1982, Larsen & Lombard 1986, Renvall 1992, Walker 1996, Pieri & Rivoire 1998), although some authors (Ryvarden 1991, Ryvarden & Gilbertson 1994) have interpreted "*Postia* Fr." as an invalid provisional name. The phylogenetic study by Yao et al. (1999) also confirmed that there is no difference between *Oligoporus* and *Postia*. Nonetheless, a subsequent phylogenetic study cited by Kotiranta et al. (2009) supported the two genera as independent, with *Oligoporus* containing species with thick-walled and distinctly cyanophilous spores. In contrast and more recently, rDNA ITS and LSU sequence analyses by Pildain & Rajchenberg (2012) place *Postia* s. lat. species in the 'postia clade' regardless of the spore wall thickness. They regard the main features of the 'postia clade' as tetrapolar mating, normal nuclear behavior, metachromatic generative hyphae, and the absence of fiber hyphae in culture. In their phylogenetic study using LSU, ITS, and combined LSU–ITS datasets, Ortiz-Santana et al. (2013) showed that the *Postia* group falls into two main clades (core postia clade, sarcoporia clade) with the 'core postia clade' comprising four subclades: /spongiporus, /oligoporus, /postia sensu stricto, and /spongiporus-undosus). They accepted *Oligoporus*, *Postia*, and *Spongiporus* as separate genera and also confirmed the independence of *Gilbertsonia*, *Rhodonia*, *Ryvardenia*, and *Taiwanofungus.*

Currently, the validity of *Postia* Fr. is generally accepted and the genus name is widely used (e.g., Buchanan & Ryvarden 2000, Wei & Dai 2006, Dai & Hattori 2007, Dai et al. 2009, Hattori et al. 2010, Yuan et al. 2010, Cui & Li 2012, Shen et al. 2014). The most recent molecular studies (Pildain & Rajchenberg 2012, Ortiz-Santana et al. 2013) place *Postia caesia* in *Postia* in the restricted sense while within *Postia* the *P. caesia* complex forms a separate branch. The phylogenies do not support the *P. caesia* group as a separate genus (as suggested by McGinty in 1909) but do support the group as a subgenus within *Postia*. Therefore the following combination is proposed:

Postia subg. ***Cyanosporus*** (McGinty) V. Papp, **comb. & stat. nov.**

MycoBank MB810903

≡ *Cyanosporus* McGinty, in Lloyd, Mycol. Notes 33: 436 (1909)

Type: *Boletus caesius* Schrad., Spic. Fl. Germ.: 167 (1794) [≡ *Postia caesia* (Schrad.) P. Karst.]

Basidiocarp medium- to small-sized, whitish when young, becoming more or less grey to blue tinted. Basidiospores narrow, small, faintly amyloid in Melzer's reagent. No chlamydospores in culture.

Species in the *Postia caesia* complex

Within the *P. caesia* complex, five species (*P. alni*, *P. caesia*, *P. luteocaesia*, *P. mediterraneocaesia*, *P. subcaesia*) are accepted in Europe (Bernicchia 2005, Niemelä et al. 2001, Pieri & Rivoire 2005, Ryvarden & Gilbertson 1994). However, other uncertain taxa described from Europe also probably belong to the *P. caesia* complex and are considered synonyms of *P. caesia* (e.g., *Bjerkandera ciliatula*, *Boletus candidus*, *B. coeruleus*, *Polyporus caesiocoloratus*).

Based on the original description (Britzelmayr 1893), *Polyporus caesiocoloratus* Britzelm. grew on a *Picea* trunk and had spores 0.7–1 µm wide. Apart from spore size, no other feature was mentioned that would distinguish the taxon from *P. caesia,* suggesting that the two species are identical.

Boletus candidus Roth is also a potential synonym of *P. caesia*, although in the original description Roth (1797) wrote that the pileus is white-yellow, with white margin and has a brown-blue pore surface.

Postia luteocaesia (A. David) Jülich is a rare Central European species, which grows exclusively on *Pinus;* its main morphological feature besides the typical blue greyish discoloration is the additional bright yellow colour of the basidiocarp (Niemelä et al. 2004, Ryvarden & Gilbertson 1994).

David (1974), who described *Tyromyces subcaesius* [≡ *Postia subcaesia* (A. David) Jülich] from deciduous trees, proved by interfertility tests that it differs genetically from *P. caesia*. With the exception its host preference, the only features separating *P. subcaesia* from *P. caesia* are the narrower spores and typically more whitish basidiocarp (Ryvarden & Gilbertson 1994).

Boletus coeruleus Schumach. (Schumacher 1803) is morphologically close to *Postia caesia* but grows on *Quercus*. Fries (1821) regarded this species as a variety of *P. caesia*, while other mycologists (e.g., Persoon 1825, Quélet 1886) considered it a distinct species. However, Bernicchia et al. (2008) reported *P. caesia* also from *Quercus cerris*, *Q. ilex*, and *Q. pubescens,* suggesting that the host is not sufficient to characterize a species.

Bjerkandera ciliatula P. Karst., described from Finland, is mentioned among the synonyms of *P. caesia* (Robert et al. 2014). However, Karsten (1887) described the type basidiocarp as small (1–1.5 cm) and growing on *Alnus incana*. These

characteristics, combined with a basidiospore width of 1 µm, suggest a closer relationship with *P. alni* than with *P. caesia*. *Postia mediterraneocaesia* M. Pieri & B. Rivoire also has a small (≤25 mm long) basidiocarp, but its pileipellis hyphae are encrusted and the basidiospores are wider (1.45–1.68 µm). It seems to be a southern European and Mediterranean species that grows on both conifers and hardwoods (Pieri & Rivoire 2005).

Tyromyces caesiosimulans G.F. Atk. was described from North America from an unknown substratum. Although Atkinson (1908) wrote that the new species resembled *Polyporus caesius* [≡ *Postia caesia*], it differs by having globose, pedicellate spores. This basidiospore shape is not known in the complex, and a type study is necessary to clarify the taxonomic position of this species.

Patouillard & Lagerheim (1892) described *Polyporus caesioflavus* from Ecuador. This species is morphologically very similar to *Postia caesia* and *P. subcaesia* (Ryvarden 1983). *Polyporus caesioflavus* is distinguished by narrower (7–9/mm) pores and a glabrous pileus surface (Loguercio-Leite et al. 2008). Carranza (1982) transferred this species to *Tyromyces* after studying the type specimen and collecting specimens from Costa Rica. Carranza's combination, which proved to be invalid (ICN, Art 41.4), was subsequently validated by Ryvarden (1983). Loguercio-Leite et al. (2008) later transferred *Tyromyces caesioflavus,* a brown-rot species (Carranza 1982), to *Oligoporus,* as *O. caesioflavus* (Pat.) Baltazar et al.

Corner (1989) described two *Tyromyces* species (*T. amyloideus*, *T. coeruleivirens*) from Malaysia (Mt. Kinabalu, Borneo), which were studied by Hattori (2002). Based on its olivaceous colour and allantoid (3–4.2 × 0.8–1 µm) slightly amyloid spores., Hattori (2002) considered *T. amyloideus* only a form of *P. caesia* s. lat., later accepting it as a synonym of *P. caesia* (Hattori et al. 2012). However, *P. caesia* s. str. has wider spores (1.3–1.7(–1.9) µm; Ryvarden & Gilbertson 1994, Niemelä 2013), suggesting that *T. amyloideus* may represent a distinct species. *Tyromyces coeruleivirens* also has a greenish pileus, monomitic hyphal system, and allantoid inamyloid basidiospores (Corner 1989, Hattori 2002). Morphologically similar to *T. amyloideus*, *T. coeruleivirens* has narrower pores (7–9/mm vs. 4–5/mm in *T. amyloideus*) and inamyloid, slightly longer (4–5 × 0.8–1 µm) basidiospores. Otherwise most characteristics (e.g., locality) are highly similar, and a taxonomic revision of these two species is needed.

Ryvarden (1988), who described *Oligoporus africanus* from Africa (Muramyya, Burundi), considered it as related to the *O. caesius* [≡ *Postia caesia*] group based on the basidiocarp type, hyphal system, and allantoid slightly amyloid spores. The main character that distinguishes *O. africanus* from *P. caesia*, *P. subcaesia*, and *P. luteocaesia* is the smaller spore size (3.5–4.5 × 1–1.2 µm) (Ryvarden 1988).

Despite the fact that *Oligoporus africanus, Polyporus caesioflavus, Tyromyces amyloideus,* and *T. coeruleivirens* have been accepted as closely related to the *Postia caesia* group ('core postia clade'), they have never been placed into *Postia.* I therefore propose the following combinations:

Postia africana (Ryvarden) V. Papp, **comb. nov.**

MycoBank MB810904

≡ *Oligoporus africanus* Ryvarden, Mycotaxon 31(2): 407 (1988)

Postia amyloidea (Corner) V. Papp, **comb. nov.**

MycoBank MB810905

≡ *Tyromyces amyloideus* Corner, Beih. Nova Hedwigia 96: 160 (1989)

Postia caesioflava (Pat.) V. Papp, **comb. nov.**

MycoBank MB810907

≡ *Polyporus caesioflavus* Pat., Bull. Soc. mycol. Fr. 8(3): 114 (1892)

Postia coeruleivirens (Corner) V. Papp, **comb. nov.**

MycoBank MB810908

≡ *Tyromyces coeruleivirens* Corner, Beih. Nova Hedwigia 96: 163 (1989)

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The new lectotypification of *Umbilicaria kisovana* (*Umbilicariaceae*, lichenized *Ascomycota*)

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Abstract — The protologue and typification of the name *Gyrophora kisovana* [*Umbilicaria kisovana*, an East Asian lichen-forming fungus] are discussed. The lectotype is designated from the Asahina collection deposited in TNS.

Key words — authorship, Japan, nomenclature, spelling, valid publication

Introduction

Umbilicaria kisovana (*Umbilicariaceae*, lichenized *Ascomycota*) is characterized by small brown brittle thalli lacking rhizinomorphs and thalloconidia, sometimes bearing gyrodisc apothecia containing octosporic asci. The distribution of the species is restricted to East Asia (Japan, Korea, Russian Far East, and China) where it grows on siliceous rocks at elevations between 400 and 2100 m (Satô 1956, Wei & Jiang 1993, Davydov & Zhdanov 2010). Wei & Jiang (1993) lectotypified the species name by an illustration published in its protologue. However, Asahina (1931) cited several syntype specimens in the protologue, and the lectotype should be selected from those specimens. The purpose of this study is to analyze the protologue and to designate the correct lectotype of *U. kisovana* from its syntypes.

Nomenclature

Asahina (1931) published *Gyrophora kisovana* (the basionym of *Umbilicaria kisovana*) with a description in Japanese. Although the protologue lacks a

Latin description, the name was accepted by Asahina and therefore is validly published according to ICN (2012: Art. 36.1(a), 38.1, 39.1).

Because the protologue was in Japanese only, there has been nomenclatural confusion. Satô (1940) provided a Latin description for *Gyrophora kisovana* based on the specimens cited by Asahina (1931) as well as several additional specimens, including a fertile specimen from Korea collected by Satô. In the Japanese summary, Satô (1940) explained the reason for providing a Latin description as, "the detailed description with illustrations of '*G. kisovana* Zahlbr.' was provided by Asahina (1931) but the Latin description has not been published yet. And also, the species was not cited in the Catalogus Lichenum Universalis. Because of such situation this species seems not to be recognized in science. Therefore, I provide the Latin description for the species."

Satô was most likely influenced by the attempts to introduce the mandatory use of Latin in earlier Codes. The Brussels Rules (1912) required Latin beginning from 1908 (Art. 35). However, the Cambridge Rules (1935), which were in effect by 1940 although probably not taken into account by Satô, moved the date to 1935 (Art. 38) – thus legalizing the new plant names in Asahina (1931). Satô (1956: 33) again published a Latin description (as "sp. nov.") citing the same reason used earlier (Satô 1940). When Zahlbruckner (1940) transferred the species into *Umbilicaria*, he cited Asahina (1931) as the source of the basionym, an authorship accepted by Llano (1950) and Wei & Jiang (1993), among others. However, Kurokawa & Nakanishi (1971) regarded Satô (1940) as the valid publication for *Gyrophora kisovana*, and proposed a new combination on that presumed basionym [i.e., *Umbilicaria kisovana* (Zahlbr. ex M. Satô) Kurok.]. Index Fungorum (www.indexfungorum.org) lists the name as *Umbilicaria kisovana* (Zahlbr. ex M. Satô) Zahlbr.

For reference purposes, we provide a translation of the complete Japanese protologue of *Gyrophora kisovana* (Asahina 1931), which may be difficult to access for most of lichenologists, along with some comments:

> «…, however a species which resembled *Heppia guepini* is distributed in Japan. I gave the Japanese common name for the species as "Hime-iwatake" [that means 'small iwatake', i.e., small *Umbilicaria esculenta* (Miyoshi) Minks], which was collected by me in Tamba Province [old district where was located in parts of Kyoto and Hyogo Prefectures, western Japan] at first. And then, this species was also collected in Nezamenotoko, along Kiso River [Nagano Prefecture] and middle slope of Mt. Nishikoma [= Mt. Nishikoma-ga-take, Kisokoma, or Kisokoma-ga-take, in Nagano Prefecture]. Hime-iwatake is slightly larger than *Heppia guepini*, however the thallus of hime-iwatake is also monophyllous and gregarious. Thallus is thin and frangible; upper surface is dark brown, and lower surface is dark blackish brown; and the thallus is attaching to granite rock by the umbilicus on the lower surface. Distinct upper and lower cortex and gonidia layer (*Pleurococcus*) are developed in the thallus. Apothecia are gyrodisc type.

> Asci are clavate, 66 × 18 µm, and 8-spored. Ascospores are ellipsoid, colorless, simple, and 16–23 × 5–7 µm in size. All of my collections were sterile but a specimen collected from Miyajima Island in Hiroshima Prefecture (leg. Atsushi Yamamoto) bear apothecia. I sent a specimen to Dr. Zahlbruckner, and he named it *Gyrophora kisovana* Zahlbr. sp. nov., but it has not yet been published.»

For the lectotype of the name *Gyrophora kisovana,* Wei & Jiang (1993) proposed the photograph of the species habit as well as the drawing of the species habit, ascus and spores that Asahina (1931) published in the protologue. However, since specimens were cited in the protologue, the lectotype must be chosen from this part of the original material (ICN 2012: Art. 9.12, 9.19 + Ex. 13).

The above translation shows that Asahina (1931) cited four collections in the protologue. They were collected from Tamba Province, Nezamenotoko along Kiso River, middle slope of Mt. Nishikoma, and Miyajima Island in Aki Province. The protologue indicates that although the first three collections were sterile, the last (from Miyajima Island) was fertile. TNS houses specimens labeled "Gyrophora kisovana" that were collected in the mentioned localities before the publication of Asahina (1931), but the specimen from Miyajima Island collected by Atsushi Yamamoto could not be found in TNS. Among Asahina's specimens, the specimen collected in Mt. Nishikoma is richly fertile, which is inconsistent with the protologue, although Dr. Asahina labeled it as "Typus" for *Gyrophora kisovana*. One of the remaining gatherings was collected at "Oninokakehashi" located in Tamba. Other specimens, separated into three packets, were collected along Kiso River in Agematsu-machi, Kiso-gun, Nagano Prefecture where "Nezamenotoko" is situated but it was not mentioned on the original label. They are morphologically consistent with the protologue and possible candidates for the lectotype. We could not find the sterile specimen on a rock, which appeared in the figure of the protologue. Among the available original material, we designate one of the specimens (TNS-L-27375) collected along Kiso River as the lectotype of *Gyrophora kisovana* (Fig. 1).

The nomenclature of the species should be treated as follows.

Umbilicaria kisovana (Zahlbr. ex Asahina) Zahlbr., Cat. Lich. Univ. 10: 405. 1940.

≡ *Gyrophora kisovana* Zahlbr. ex Asahina, J. Jap. Bot. 7: 327. 1931.

Type: Japan. Honshu. Prov. Shinano (Pref. Nagano): along Kiso River, Nezamenotoko, Agematsu-machi, Kiso-gun, on granite rocks, elevation about 700 m, 17 October 1927, Y. Asahina s.n. (**Lectotype designated here**, TNS-L-27375; **isolectotypes**, TNS-L-27389, TNS-L-27390).

Other syntype traced: Japan. Honshu. Prov. Tamba (Pref. Hyogo): Oninokakehashi, Hikami-gun, 14 July 1927, Y. Asahina s.n. (TNS-L-27393).

Asahina did not directly explain the etymology of *Gyrophora kisovana* in the protologue, but the epithet was apparently derived from the locality, Kiso. However, some of the original labels signed by Asahina read "*Gyrophora*

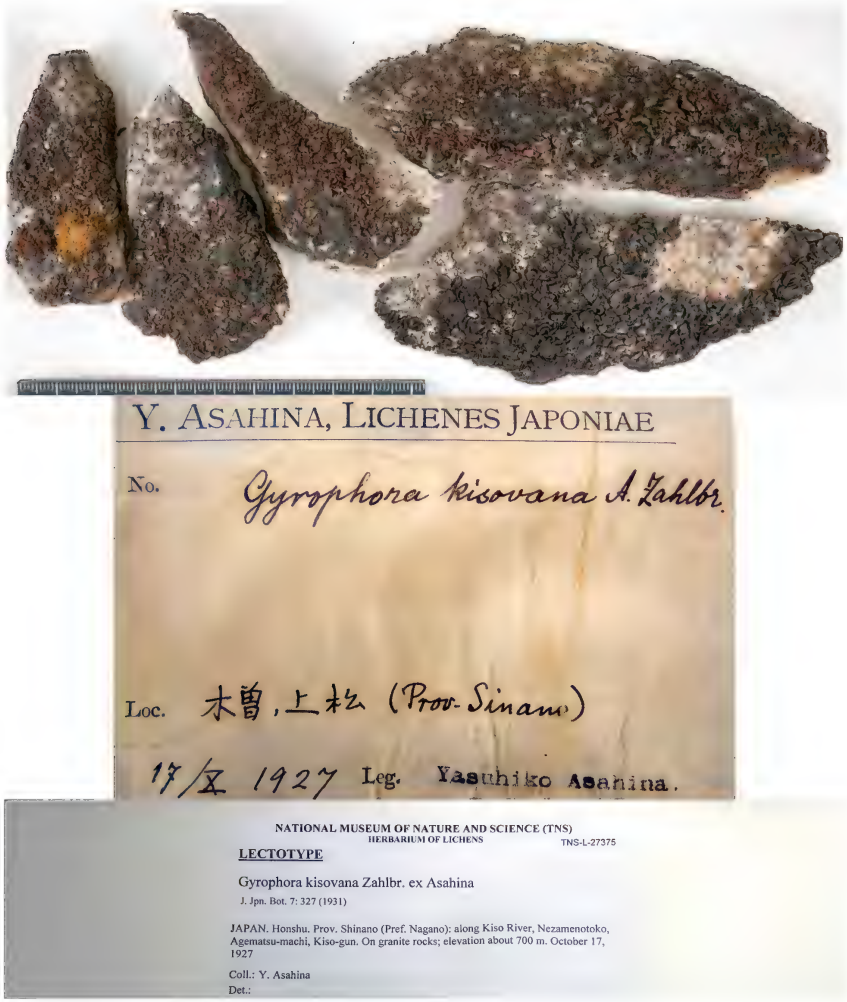
Y. ASAHINA, LICHENES JAPONIAE
No.
Gyrophora kisovana A. Zahlbr.
Loc. 木曽，上松 (Prov. Sinano)
17/X 1927 Leg. Yasuhiko Asahina.
NATIONAL MUSEUM OF NATURE AND SCIENCE (TNS)
HERBARIUM OF LICHENS
TNS-L-27375
LECTOTYPE
Gyrophora kisovana Zahlbr. ex Asahina
J. Jpn. Bot. 7: 327 (1931)
JAPAN. Honshu. Prov. Shinano (Pref. Nagano): along Kiso River, Nezamenotoko, Agematsu-machi, Kiso-gun. On granite rocks; elevation about 700 m. October 17, 1927
Coll.: Y. Asahina
Det.:

FIGURE 1. *Gyrophora kisovana* (lectotype, TNS-L-27375).

kisoana" (TNS-L-27390) or "*Gyrophora kisogawana*" (TNS-L-27393). These provisional names have never appeared in the protologue or any other publication, however, and the validly published original orthography "*kisovana*" must be accepted (ICN 2012: Art. 60.1).

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We acknowledge Dr. A. Sennikov (Finnish Museum of Natural History, University of Helsinki) for his valuable comments, Dr. S. Chabanenko (Sakhalin Botanical Garden)

for technical help, and Prof. B. McCune (Oregon State University) for improving the text.

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Galerella xalapensis sp. nov. found in an urban green area in Xalapa, Veracruz, Mexico

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Abstract — While monitoring species diversity of urban green areas in Xalapa city (Veracruz, Mexico), a new species of *Galerella* was found in an abandoned urban green area. After macro- and microscopic analysis it is proposed as a new species, *Galerella xalapensis*, characterized by a faintly plicate-sulcate dry pale grayish brown pileus, white to yellowish glabrous stipe, thick-walled rusty-brown ellipsoid basidiospores, utriform cheilocystidia, and clampless hyphae. The new taxon is described and illustrated, and a comparison with similar species is provided. This is the first *Galerella* recorded from Mexico.

Key words — *Agaricales*, *Bolbitiaceae*, biodiversity, neotropical fungi, taxonomy

Introduction

A core of agaricoid species within the *Bolbitiaceae* (*Agaricales*), related to *Bolbitius* Fr., *Conocybe* Fayod, and *Pholiotina* Fayod, has been recognized as the genus *Galerella* Earle. Although supraspecific relationships derived from molecular phylogenetic analyses have not yet been fully explored, the striking combination of morphological features of the taxa accommodated in *Galerella* led to their characterization by a *Coprinus*-like dry and plicate-sulcate pileus, hymeniform pileipellis, rust-colored basidiospores, and lageniform cheilocystidia (Horak 1968, Pegler 1986, Singer 1986, Hausknecht & Contu 2003). We refer especially to the works of Horak & Hausknecht (2002), Arnolds & Hausknecht (2003), Hausknecht & Contu (2003), and Hausknecht et al. (2004), who not only described new species but also reevaluated the taxonomy of the genus through type studies of earlier named species and integrated informative taxonomic characters to recognize six species currently in *Galerella*. The literature shows that *Galerella* species are rare, but distributed worldwide (Fig. 1). Two widespread species include *G. plicatella* (Peck) Singer, described from USA and reported from Argentina, Brazil, India, Italy, and

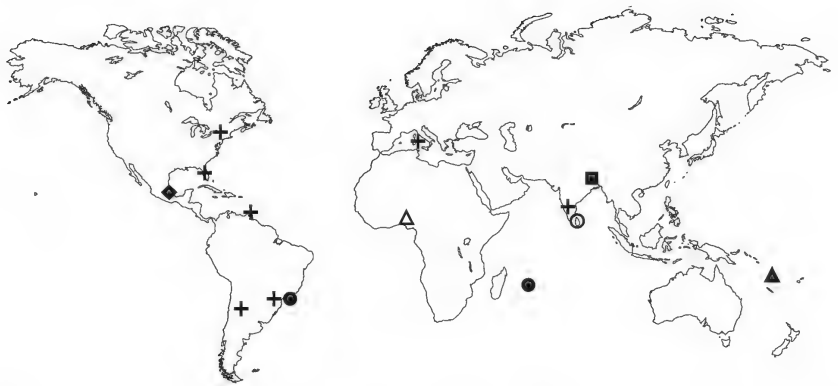

FIGURE. 1. Representation of the worldwide distribution of *Galerella* species according reports in litt. *G. fibrillosa* (•). *G. floriformis* (▲). *G. microphues* (○). *G. nigeriensis* (△). *G. plicatella* (+). *G. plicatelloides* (▪). *G. xalapensis* (♦).

Trinidad (Dennis 1953, Singer & Digilio 1951, Thomas et al. 2001, Horak & Hausknecht 2002, Hausknecht & Contu 2003, Arnolds & Hausknecht 2003, Hausknecht et al. 2004) and *G. fibrillosa* Hauskn., described from Mauritius and recorded from Brazil (Horak & Hausknecht 2002); but known only from their type localities are *G. floriformis* Hauskn. from Vanuatu, *G. microphues* (Berk. & Broome) Pegler from Sri Lanka, *G. nigeriensis* Tkalčec et al. from Nigeria, and *G. plicatelloides* Sarwal & Locq. from India (Sarwal & Locquin 1983, Pegler 1986, Hausknecht & Contu 2003, Tkalčec et al. 2010).

Recently a bolbitiaceous fungus was found that turned out to represent *Galerella*, a genus previously unrecorded from Mexico. The site is in an abandoned green area, one of multiple points previously established to monitor species diversity of the vascular plants, macrofungi, ants, butterflies, beetles, amphibians, and birds that inhabit urban green areas in Xalapa city (Veracruz, Mexico), a long-term program currently being conducted by a research team of the Instituto de Ecología. After a thorough morphological examination, we concluded that the specimen's unique combination of characters (faintly plicate-sulcate dry pale grayish brown pileus, white to pale yellowish glabrous stipe, thick-walled ellipsoid basidiospores, utriform cheilocystidia, clampless hyphae) confirmed it as an undescribed species of *Galerella*.

Materials & methods

As part of the monitoring program developed in urban green areas from Xalapa city, once a week during June–September 2012 we visited the intra-urban point located at 1230 m alt. south east of Xalapa that corresponds to a heavily disturbed patch of forest formerly used as an orchard. After collecting the single *Galerella* sample in July 2012, we

sampled this point of our sampling mesh daily during May–October 2013 to document thoroughly the fruiting variation of this *Galerella* species. The macroscopic depiction in Pl. 1 is based on field specimens. Macroscopic characters are described from material and microscopic characters were based on dried basidiomata. Color descriptions are according to Kornerup & Wanscher (1967). Tissue sections were mounted in 3% KOH or 1% Congo Red aqueous solution for microscopical examination according to Largent et al. (1977). 110 basidiospores (55 in lateral view and 55 in frontal view) were measured in KOH, and 35 cheilocystidia in Congo Red, basidiospore measurements correspond to length × width in side view × width in frontal view; X = mean length & width values; Q = the mean length/width ratio. Line drawings were made under the microscope, with the aid of a drawing tube. The examined specimen is deposited at XAL herbarium (Thiers B., Index Herbariorum: http://sweetgum.nybg.org/ih/).

Taxonomy

Galerella xalapensis Bandala & Montoya, **sp. nov.** Plate 1

MycoBank MB 809145

Differs from other similar species of *Galerella* by its pale grayish brown pileus, white glabrous stipe, narrowly utriform to utriform cheilocystidia, and absence of clamp connections.

Holotype: Mexico. Veracruz: Xalapa, 26 July 2012, Corona 699 (XAL).

Etymology: the epithet refers to Xalapa, the city of origin.

Basidioma slender, delicate, moderately small-sized. Pileus 48–50 mm broad, at first probably broadly convex, becoming plane-depressed or plane-concave, with a weakly raised knob at center, surface pale grayish brown (5D8) irregularly interrupted by paler, almost whitish radial lines, with a darker contrasting area rounding the center, dull, dry, not hygrophanous, faintly plicate-sulcate (*Coprinus*-like) from the disk towards the margin, this latter somewhat undulating. Lamellae narrowly adnate, close, ventricose, whitish to pale grayish, with paler, weakly fimbriate edges. Stipe 150 × 1–1.5 mm, cylindric, almost straight, white, in parts pale yellowish (2.5 Y 8/2), finely and weakly striate, glabrous, dry, fistulose. Context (pileus) very thin (<1 mm thick), whitish (pileus and stipe); odor and taste not distinctive.

Basidiospores 8–11.5(–12.5) × 4.5–6 × 5.5–7(–7.5) µm, ellipsoid in side view, $X = 9.5 \times 5.3$ µm, $Q = 1.80$ µm, broadly ellipsoid, often slightly tapering towards apex and weakly angular at base, then somewhat subhexagonal or subamygdaliform in dorso-frontal view, $X = 9.6 \times 6.1$ µm, ($Q = 1.58$ µm), at times the adaxial side faintly depressed then more or less reniform, thick-walled (<1 µm thick), smooth, with central, more or less truncate germ-pore, reddish-orange, inamyloid, not-dextrinoid. Basidia 16–25 × 8–11 µm, clavate to broadly clavate, 4-spored, some 3-spored, hyaline, thin-walled. Pleurocystidia and Pileocystidia absent. Cheilocystidia 24–48(–55) × 7–20(–22) µm, apex 5–13 µm broad, narrowly utriform to utriform, often

ventricose or with a flexuous neck, occasionally sublageniform, thin-walled, smooth, hyaline, scarce on lamellae edge. Pileipellis hymeniform, composed of clavate to broadly clavate elements 18–35(–38) × 7–12(–13) µm, thin-walled, smooth, easily collapsing, hyaline or with a pale grayish content then in mass the layer appears irregularly pale brownish, inamyloid. Hymenophoral trama regular, made of cylindrical hyphae tightly arranged, hyaline, thin-walled. Pileus trama not recovered, collapsed. Caulocystidia 21–41 × 6–9 µm, more or less similar to cheilocystidia, scarce, occasionally in small groups. Clamp connections absent.

Habitat — Solitary on naked soil, in a small slope of the floor of a green heavily disturbed urban forest patch, with scattered trees (*Eriobotrya japonica* (Thunb.) Lindl., *Fraxinus uhdei* (Wenz.) Lingelsh., *Psidium guajava* L., and *Platanus mexicana* Moric.), shrubs (*Bunchosia lindeniana* A. Juss. and *Solanum nudum* Dunal), and herbs (*Hypoxis decumbens* L., *Paspalum conjugatum* P.J. Bergius, *Acalypha alopecuroides* Jacq., *Desmodium uncinatum* (Jacq.) DC., *Cyperus hermaphroditus* (Jacq.) Standl., and *Rivina humilis* L.).

Distribution — Known only from the type locality in Xalapa.

Remarks — The unique combined set of characters such as the faintly plicate-sulcate dry pale grayish brown pileus, thick-walled ellipsoid basidiospores, narrowly utriform to utriform cheilocystidia, and clampless hyphae distinguish *Galerella xalapensis*. To a certain extent, its habit resembles *G. microphues*, *G. plicatella,* and *G. fibrillosa* but the Mexican species differs strikingly in remarkable important features. *Galerella microphues*, a rather uncommon species restricted to Sri Lanka, is distinguished by narrowly lageniform cheilocystidia (22–35 × 7–9 µm) with a long tapering neck (10–20 × 1.5–2 µm), pyriform (16–25 × 11–16 µm) pileipellis elements, and clamped hyphae (Pegler 1986). The most frequently encountered species (per Horak 1968, Hausknecht & Contu 2003, Hausknecht et al. 2004, Thomas et al. 2001), *G. plicatella* is easily diagnosed by its brown pileus with alternating regions of pale orange, yellowish or fulvous-brown that turns alutaceous-buff with an orange-apricot disc, pale orange lamellae, finely pubescent orange-white stipe, ventricose or lageniform cheilocystidia with a remarkably slender long neck and measuring 30–50 × 6–11 µm, 16.5–54 × 8–16.5 µm, or 20–50 × 6.5–10 µm, and clamped hyphae. *Galerella fibrillosa* — known only from the holotype collected on soil in Mauritius and another specimen from Brazil gathered on a decayed twig — is readily separated by its small fragile basidiomata (pileus 7–15 mm; stipe 30–40 × 0.5–0.8 mm), a conspicuously crenate and fibrillose pale yellow-brown pileus with yellowish ochre or pale brown disc, rapidly deliquescent pale brown to yellowish brown lamellae, vesiculose cheilocystidia (28–)32–55(–62) × 10–20 µm with more or less distinctive papilla to lageniform with elongate cylindrical

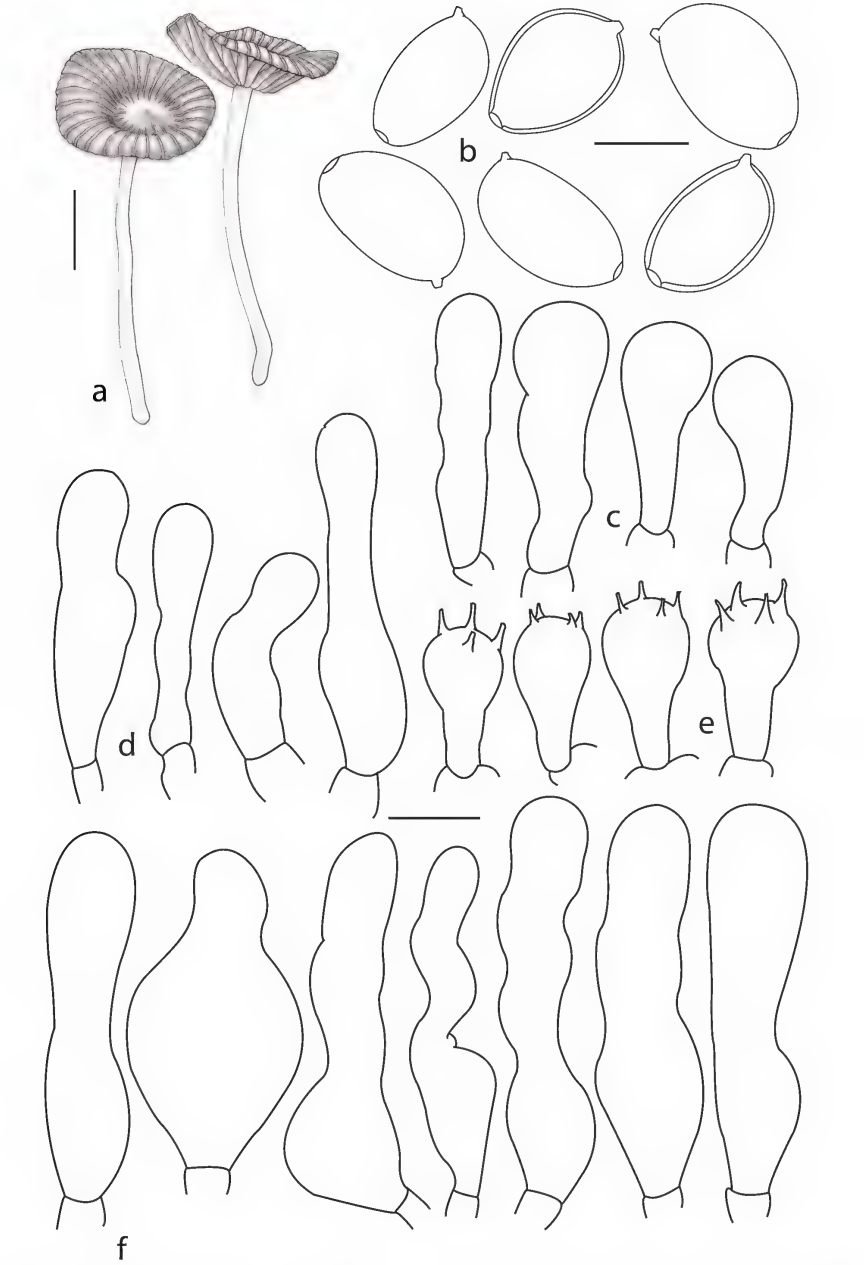

Plate 1. *Galerella xalapensis* (holotype, XAL Corona 699). a. Basidiomata. b. basidiospores. c. elements of pileipellis. d. caulocystidia. e. basidia. f. cheilocystidia. Bars: a = 25 mm; b = 5 µm; c–f = 10 µm.

neck, and clamped hyphae (Horak & Hausknecht 2002). The remaining three known *Galerella* species also differ: *G. plicatelloides* and *G. floriformis* can be recognized by their fertile lamellae edges (i.e., cheilocystidia absent) and *G. nigeriensis* by its strongly plicate-sulcate pale yellowish brown to light orange brown pileus, pubescent stipe, tibiiform to lageniform cheilocystidia, and presence of hymenophysalides (Sarwal & Locquin 1983, Hausknecht & Contu 2003, Tkalčec et al. 2010).

It is important to mention that *G. xalapensis* may have a particular fruiting pattern, judging by the behavior observed in the sampled site. After a single collection appeared in July 2012, we increased our sampling frequency to once a week during August–September 2012, during which we found no basidiomata of *G. xalapensis*. Although we visited the site daily during May–October 2013, we never again observed specimens of this *Galerella* species. The question is whether *G. xalapensis* represents a fungal group that fluctuates in occurrence and abundance, not fruiting for several consecutive months or years but remaining in place as a physiologically active mycelium, or whether it suffers from disturbance, so that finding a specimen at the site was a matter of chance and good fortune. A similar example is represented by *G. nigeriensis*, which was collected at the edge of a heavily disturbed secondary tropical forest (Tkalčec et al. 2010). Given that we continue to monitor the study site thoroughly and recognizing that the specimen combines a striking set of taxonomic characters, we decided to propose the new species here. Nonetheless, we will continue to monitor the type locality for *G. xalapensis* and other agaricaceous fungi as part of our sampling program in Xalapa's urban green areas.

Acknowledgments

We thank INECOL for the financial seed grant to support the monitoring program in the urban green areas in Xalapa. We appreciate the collaboration in the monitoring of macrofungi in the field of the biologists J.C. Corona and D. Ramos, this latter also assisted us during the microscopic study. Special thanks to E. Saavedra for elaborating the line drawing of the basidiomata and Dr. L. Lorea for the plants information from the site studied (all at INECOL). We acknowledge the valuable revision and comments on the manuscript by Dr. A. Hausknecht (Austrian Mycological Society) and Dr. I. Krisai-Greilhuber (University of Vienna). Especial thanks to Dr. S. Pennycook and Dr. L. Norvell for their meticulous revision and improving the text.

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A new report of *Uromyces ambiens* on *Buxus* from Pakistan

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Abstract — The rust fungus, *Uromyces ambiens,* is described and illustrated based on a specimen of *Buxus wallichiana*, Himalayan boxwood, from northern Pakistan. This rare fungus is reported for the first time from this host and for the first time from Pakistan.

Key words — dimorphic spores, Himalayas, *Puccinia buxi*, Shangla

Introduction

During the exploration of *Uredinales* of District Shangla, Khyber Pakhtunkhwa, Pakistan, trees of Himalayan boxwood, *Buxus wallichiana*, were found to be infected with a species of *Uromyces*. Leaves of *B. wallichiana* collected in September 2013 were infected with pale yellowish spermogonial sori. Later samples collected in November and January showed both spermogonial and telial sori. After an extensive literature survey, we identified the rust fungus on *Buxus* as *Uromyces ambiens*.

Another rust fungus, *Puccinia buxi* Sowerby, known as boxwood rust, occurs on many species of *Buxus*. It is widespread throughout Europe but rarely reported on native hosts in China and Japan. The boxwood rust has been intercepted at ports of entry in North America over the past decades and was discovered in Pennsylvania on recently received nursery stock of boxwood from Greece (Yun 2014).

Himalayan boxwood is a medium sized small tree found commonly in the western and central Himalayas at elevations ranging from 1200 to 2900 m (Viswanath et al. 2006). The tree is native to Pakistan and India. In Pakistan, it grows in the sub-Himalayan tract from Azad Kashmir westward to Rawalpindi, Islamabad, Murree, Hazara, Chitral, Swat, Shangla, Rawlakot, and the Gadoon area (Stewart 1972).

Our collections of *Uromyces ambiens* represent a new record for Pakistan as well as a new host record. Pakistan is the second country in which this rare rust on *Buxus* occurs. In addition, this represents the only recent re-description and illustration of this species.

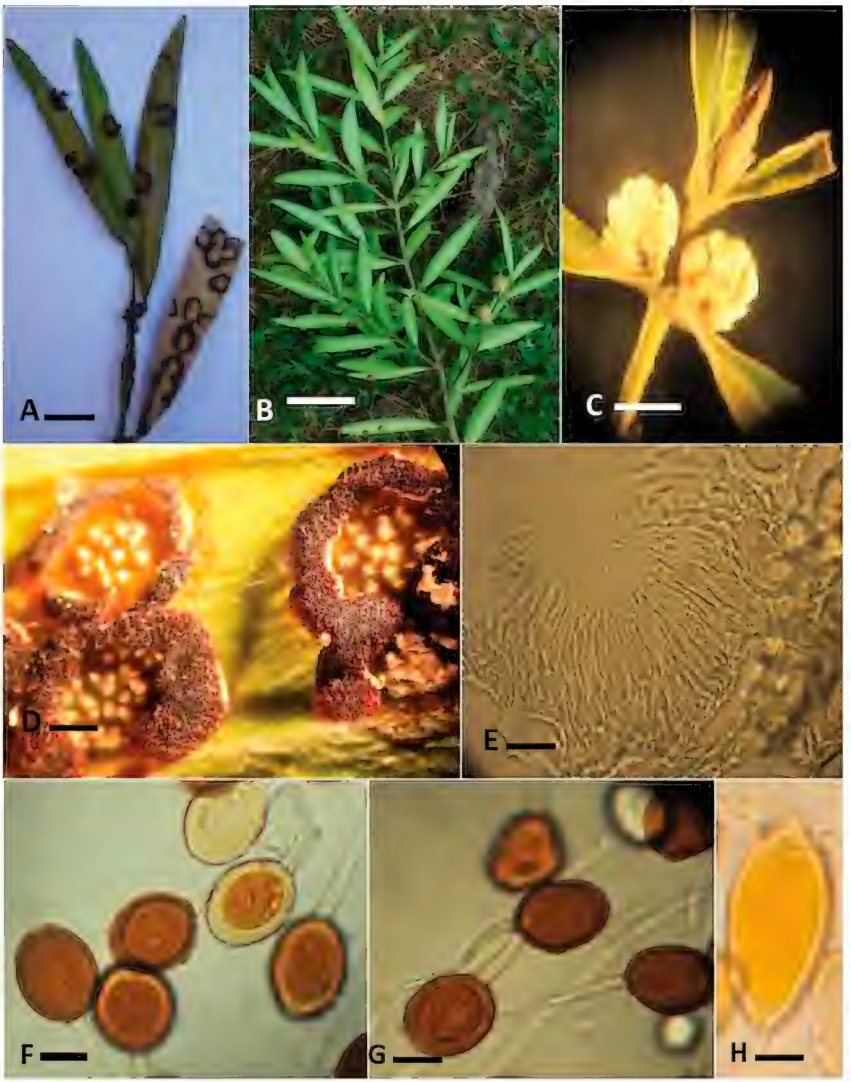

Plate 1: *Uromyces ambiens*. A, B: Leaves of *Buxus wallichiana* showing raised spermogonial and telial sori. C: Inflorescence of *Buxus wallichiana*. D: Telial sori under stereomicroscope. E: Longitudinal section of spermogonium. F–H: Dimorphic teliospores. Scale bar = 10 µm.

Materials & methods

Freehand sections and scrape mounts of infected plant materials were made in lactic acid. The plant was photographed and infected portions were observed under a stereomicroscope. Twenty spores of each spore state were examined under a microscope (Nikon YS 100) and measured using an ocular micrometer (Zeiss Eyepiece Screw Micrometer). Sections and spores were photographed by Digiporo-Labomed. The spores were illustrated with the aid of a camera lucida (Ernst Leitz Wetzlar Germany).

Taxonomy

Uromyces ambiens Cooke, Grevillea 3: 75 (1874) PLATES 1, 2

AECIA and UREDINIA not found. SPERMOGONIA subepidermal, group 1, type 1, golden brown to hyaline, 175 × 137 µm. TELIA abaxial, raised, developing as circular black boundaries around central yellow spots, scattered, naked, chestnut brown, 2–3 × 4–5 mm. TELIOSPORES dimorphic: ovate teliospores yellow, elongated, 1-celled, 29–40 × 48–60(–79), walls smooth, yellow; globose to subglobose teliospores brown, 29–47 × 39–48 µm, walls smooth and dark

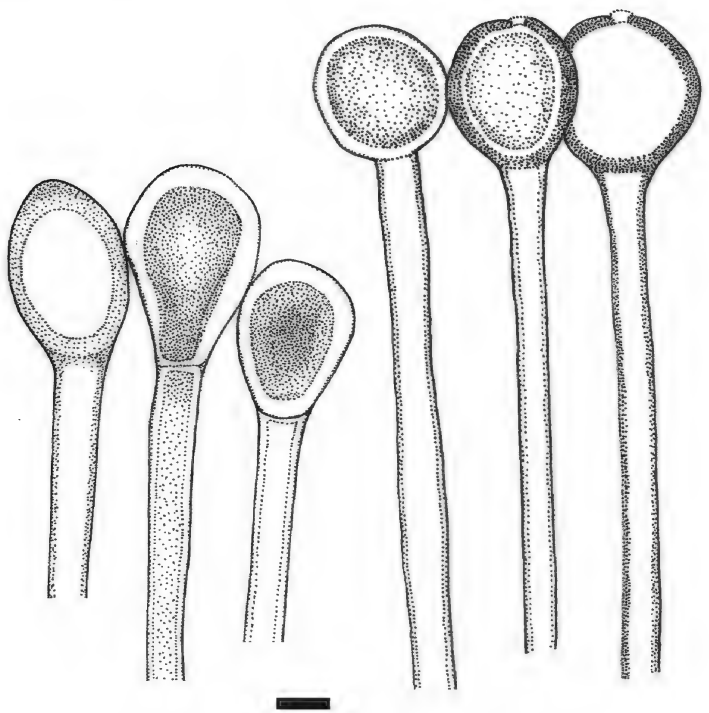

PLATE 2: *Uromyces ambiens:* teliospores. Scale bar = 10 µm.

brown, 2.8–5 µm thick at sides, up to 3.5 µm thick apically, germ pore one, subapical to apical; pedicel hyaline, 6.5–9 × 160–205(–285) µm.

MATERIAL EXAMINED: **PAKISTAN**, **KHYBER PAKHTUNKHWA**, Shangla district, Yukhtange, at 2200 m a.s.l., on *Buxus wallichiana* Baill. (*Buxaceae*), stages 0 + III, 2 Sep. 2013, A.N. Khalid AM-S1 (LAH 20914); 4 Nov. 2013, Sadiqullah RS 36A (LAH 20913A); 1 Jan. 2014, Sadiqullah RS 36B (LAH 20913B).

COMMENTS: Cooke (1874) originally described *Uromyces ambiens* on *Buxus sempervirens* L. from the Indian Himalayas, and Sydow (1922) published a revised and expanded description of the type collection. Later Butler & Bisby (1931) reported this species on leaves of *Buxus* (probably *sempervirens*) near Dunooltie above Dehra Dun as well as from Bashahr, near Simla, 6000 ft. elevation. Although Spaulding (1961) listed *U. ambiens* as present in India and Pakistan, we found nothing in the Pakistani literature about this rust.

Uromyces ambiens differs morphologically from *Puccinia buxi.* In *U. ambiens* the teliospores are dimorphic (ovate or globose to subglobose), non-septate, and thick-walled (up to 5 um thick on the sides), while *P. buxi* has only monomorphic teliospores that are oblong to clavate, one-septate, and thin-walled.

Acknowledgements

We are sincerely thankful to Dr. Amy Rossman (Systematic Mycology and Microbiology Laboratory, USDA-ARS, Beltsville) and Dr. Abdul Rehman Niazi (Department of Botany, University of the Punjab, Lahore, Pakistan) for acting as presubmission reviewers and giving valuable suggestions.

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Lophium elegans (*Ascomycota*), a rare European species

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Abstract — *Lophium elegans* (*Mytilinidiales, Mytilinidiaceae*) is recorded for the first time in northern Europe. It was found on *Juniperus communis* in two locations in Finnmark County, Norway, which represent the northernmost finds for the species. A morphological description of the species is given and its ecology and distribution discussed.

Key words — host preference, junipericolous fungi

Introduction

Lophium elegans is an ascomycete known to science for about 60 years (Zogg 1954) but collected only a few times by a small number of mycologists. The species has been found only in Europe where it has been reported from rather high altitudes in southern France (Alpes Maritimes, Hautes Alpes), northern Italy (South Tyrol), Switzerland, and Scotland (Zogg 1962, Kirk & Spooner 1984). The finds from southern Europe were all made between 1940 and 1960 (Zogg 1962), and the two finds from Great Britain date back to the beginning of the 1980s (Kirk & Spooner 1984). In 2008, the species was collected from near Dijon, in the Burgundy region of east central France (Lechat 2013).

Lophium elegans seems to be restricted to *Juniperus*. In Great Britain and France the species was found on *J. communis*, and in Italy and Switzerland on *J. communis* ssp. *nana* [as *J. nana*] (Zogg 1962, Kirk & Spooner 1984, Lechat 2013). Apart from *Lophium igoschinae* Chleb., found on *Dryas* (*Rosaceae;* Chlebicki & Knudsen 2001), all *Lophium* species have been collected only from coniferous substrates (Zogg 1962, Boehm et al. 2009). Zogg (1962) reported *Lophium elegans* as growing on bark of small living and dead twigs of its host tree, while Ellis & Ellis (1997) reported it on wood of *J. communis*.

Here we report three records of *L. elegans* from Northern Norway, where the species was found on *J. communis* in two different locations. These represent the first northern European records of the species.

Materials & methods

Lophium elegans was collected during ascomycete field inventories in Finnmark, Norway, 2010 and 2011. Geographical coordinates were taken in the field using GPS. The collections were studied using Wild M10 and Zeiss 475052-9901 dissecting microscopes and Leitz DMRBE and Zeiss 473028 light microscopes. Microscopic slides were prepared from dried herbarium material and mounted in water for measurements and photographs. Photographs were taken with Olympus UC30 and UC50 digital cameras. Collector abbreviations include GM (Mathiassen) and AG (Granmo). Specimens are deposited in the herbarium of Tromsø University Museum, Norway (TROM).

Taxonomy

Lophium elegans H. Zogg, Ber. Schweiz. Bot. Ges. 64: 141 (1954) Fig. 1

Ascomata 200–500 µm high, 180–340 µm wide, and 90–120 µm thick, seated upright on bark or erumpent through cracks in bark, scattered or clustered, laterally flattened and often striate, usually axe-head shaped (dolabriform), somewhat tapering towards base, with a long slit-like ostiolum along the ± sharp upper edge, brittle and black. Asci 160–210 × 7.2–8.7 µm, cylindrical, bitunicate, short stiped, containing 8 spirally entwined spores. Paraphysoids ca. 1.5 µm diam., long, branched, anastomosing, septate and hyaline. Ascospores 210–265 µm × 1.3–2 µm, filiform, tapered at ends, multiseptate with septa at intervals of mostly 5–8 µm, yellowish hyaline, gradually becoming spirally arranged within the ascus.

Specimens examined: **NORWAY, Finnmark: Municipality of Alta**, the valley Eibydalen, 69°46'30"N 23°18'49"E, 217 m asl, on dead twig of *Juniperus communis* L., 17.VIII.2011, G. Mathiassen & A. Granmo, GM 12472 C (TROM F-41138); 69°46'31"N 23°18'28"E, 204 m asl, on dead twig of *J. communis*, 17.VIII.2011, A. Granmo & G. Mathiassen, AG 25A/2011 (TROM F-25034). **Municipality of Karasjok**, Basavzeguoika, in the upper, southern part of the Anarjohka river valley, 53°20'689" N 33°22'29" E, 205 m asl, on dead twigs of *J. communis*, 18.VIII.2010, G. Mathiassen & A. Granmo, GM 12297 A (TROM F-25741).

Discussion

Lophium elegans was found on *Juniperus communis* at Basavzeguoika 205 m asl, in the Anarjohka river valley, Karasjok, in August 2010. This area is a continental part of Northern Norway with relatively high summer temperatures (mean of the warmest month, July, 13.1°C; DNMI 1991) and low precipitation (interpolated annual precipitation 366 mm; Moen 1998). The species was also found twice in 2011 in the valley Eibydalen in Alta. The Alta area is an inner fjord district in the middle boreal zone with luxuriant woodland and

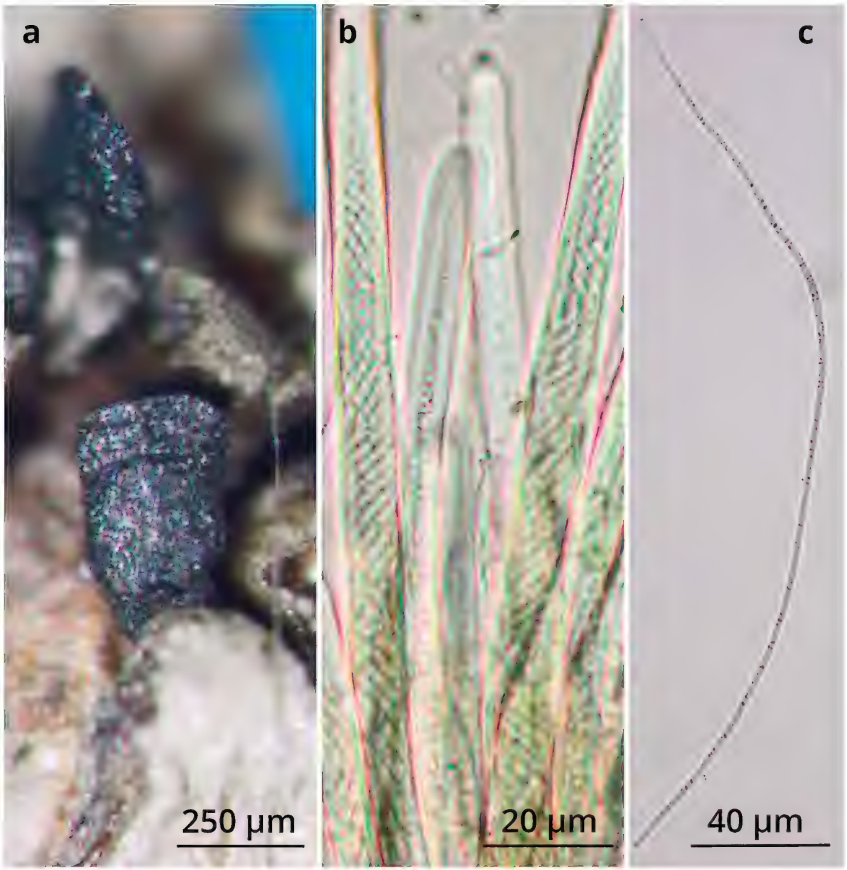

Fig. 1. *Lophium elegans*: a. Ascomata (GM 12297 A). b. Asci containing ascospores (GM 12472 C). c. One ascospore (GM 12472 C). Photos: GM (a-b), TR (c).

warm summers (mean of the warmest month, July, 13.4°C; DNMI 1991). Our first find at Basavzeguoika and the world's northernmost finds in Eibydalen represent the first records of *L. elegans* from northern Europe despite earlier searches by the renowned Swedish ascomycete researchers Kerstin and Lennart Holm. They were well aware of this species, but did not find it in any of the Nordic countries (Holm & Holm 1977). The species has a disjunctive distribution pattern on the European mainland (Fig. 2), similar to that of a few other pyrenomycetous species, e.g., *Glyphium grisonense* Math., *Hypoxylon macrosporum* P. Karst., and *Saccardoella kanderana* Math. (Mathiassen 1989, 1993; Granmo 1999; Mathiassen & Granmo 2012). Therefore, as *L. elegans* is known from Scotland, it is likely that some of those species may also occur at

Fig. 2. World distribution of *Lophium elegans*. The large dot represents 6 localities along the Alpes Maritimes, where Zogg collected the species in 1955 and 1960. Map: E. Høgtun ©, Tromsø University Museum, 2013.

high altitudes in the northern British Isles. Although *L. elegans* is undoubtedly a rather rare species in Europe, we expect it to occur occasionally along the

whole Scandinavian mountain range as well as in other high altitude localities along the Alps, e.g., in Austria and southern Germany.

The Norwegian records strengthen the impression of *L. elegans* as being restricted to *Juniperus,* which we consider a junipericolous fungus. The three Norwegian specimens had ascocarps growing on bark as well as on rather small twigs. Nevertheless, one single ascocarp was observed growing on a needle, which has never been reported earlier (cf. Kirk & Spooner 1984). Because of this and Ellis & Ellis' (1997) observation from naked wood, *L. elegans* should be considered not as a strictly, but rather a predominantly, corticolous species.

The ascospores of *L. elegans* are very long and arranged in a spiral parallel configuration in the asci, which Zogg (1954) termed "parallel-spiralig aufgerollte" spores. However, Kirk & Spooner (1984) maintained that this character may be exhibited only at maturity, in that ascospores in the British collections lay parallel in young immature asci and spirally coiled in mature asci. One Norwegian sample (AG 25A/2011) supports this observation. *Lophium elegans* is morphologically rather similar to *L. mytilinum* (Pers.: Fr.) Fr., which differs by its longer, thicker ascocarps (Zogg 1962). In addition, *L. mytilinum* ascospores always lie parallel in, and remain shorter than, the asci (Zogg 1962). Kirk & Spooner (1984) pointed out that *L. elegans* was evidently very closely related to *L. mytilinum* but distinguished particularly by the spiral coiling of the ascospores. However, molecular data indicate that the two species are not closely related within the family *Mytilinidiaceae* (Boehm et al. 2009), and that *Lophium elegans* and *L. mytilinum* might, in fact, be placed in two separate genera in that family. According to Boehm et al. (2009) both *Lophium* and *Mytilinidion* are polyphyletic. However, until the type species of *Mytilinidion*, *M. aggregatum* (DC.: Fr.) Duby, and type species of other genera in *Mytilinidiaceae* are included in a common phylogenetic analysis with taxa mentioned by Boehm et al. (2009), any new nomenclatural combinations can only be speculative.

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New and interesting *Laboulbeniales* from southern and southeastern Asia

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Abstract — Two new species of *Laboulbenia* from the Philippines are described and illustrated: *Laboulbenia erotylidarum* on an erotylid beetle (*Coleoptera*, *Erotylidae*) and *Laboulbenia poplitea* on *Craspedophorus* sp. (*Coleoptera*, *Carabidae*). In addition, we present ten new records of *Laboulbeniales* from several countries in southern and southeastern Asia on coleopteran hosts. These are *Blasticomyces lispini* from Borneo (Indonesia), *Cantharomyces orientalis* from the Philippines, *Dimeromyces rugosus* on *Leiochrodes* sp. from Sumatra (Indonesia), *Laboulbenia anoplogenii* on *Clivina* sp. from India, *L. cafii* on *Remus corallicola* from Singapore, *L. satanas* from the Philippines, *L. timurensis* on *Clivina inopaca* from Papua New Guinea, *Monoicomyces stenusae* on *Silusa* sp. from the Philippines, *Ormomyces clivinae* on *Clivina* sp. from India, and *Peyritschiella princeps* on *Philonthus tardus* from Lombok (Indonesia).

Key words — *Ascomycota*, insect-associated fungi, morphology, museum collection study, Roland Thaxter, taxonomy

Introduction

One group of microscopic insect-associated parasitic fungi, the order *Laboulbeniales* (*Ascomycota, Pezizomycotina, Laboulbeniomycetes*), is perhaps the most intriguing and yet least studied of all entomogenous fungi. *Laboulbeniales* are obligate parasites on invertebrate hosts, which include insects (mainly beetles and flies), millipedes, and mites. About 80% of the *Laboulbeniales* described thus far are parasitic on the order *Coleoptera* (Weir & Blackwell 2005). With currently about 2,000 species in 140 genera (Rossi & Santamaría 2012), the total number of *Laboulbeniales* associated with arthropod hosts is estimated to be between 15,000 and 75,000 species (Weir &

Hammond 1997), most of which still need to be described from wet tropical regions (Haelewaters et al. 2014a).

This paper presents ten new records for different southern and southeastern Asian countries. Additionally, we report thalli in the genus *Laboulbenia* that did not fit any existing description and therefore represent two new species.

Materials & methods

Over 23,000 insect specimens in the collection of Invertebrate Zoology at the American Museum of Natural History (AMNH) were screened for the presence of entomopathogenic fungi using a Nikon SMZ-U stereomicroscope. Almost six percent of these insects were found infested with *Laboulbeniales* (Haelewaters unpublished). Although the vast majority of the entomological collection at AMNH is the result of samplings in North America, a few infected hosts from Asia were found. Individual thalli were removed from the host's integument with a Minuten Pin (BioQuip #1208SA) and embedded in Amann solution or PVA Mounting Medium (BioQuip #6371A). Cover slips were ringed with transparent nail varnish. All microscopic slides are deposited at the Farlow Herbarium, Harvard University (FH).

Additional fungal material was found in unidentified mountings from Roland Thaxter, whose complete collection is deposited at the Farlow Herbarium, Harvard University (FH).

Morphological analyses, measurements, and photographs were made using an Olympus BX40 light microscope with Olympus XC50 digital camera and MicroSuite Special Edition software 3.1 (Soft Imaging Solutions GmbH). Images were optimized (with Levels and Brightness/Contrast tools) and cropped in Adobe Photoshop CS Version 8.0 (San Jose, California).

Taxonomy

Blasticomyces lispini (Thaxt.) I.I. Tav., Mycol. Memoir 9: 155 (1985) Plate 1a

Specimen examined: **INDONESIA, Borneo**, Central Kalimantan Prov., Tanjung Puting National Park, on "staphylinid close to *Lispinus*" [sic] (*Staphylinidae*, *Osoriinae*), 1926, leg. Eric Mjöberg, *Thaxter 3571*, slide FH 00313486 (4 thalli collected from elytra).

Previous records: *On Nacaeus impressicollis* [as *Lispinus*] and various *Lispinus* sp. from Indonesia (Java), Taiwan, Sri Lanka, the Philippines, China, Malaysian Borneo, and the Solomon Islands (Thaxter 1915, 1931, Majewski & Sugiyama 1986).

Three species are included in *Blasticomyces* I.I. Tav. All three have been previously observed on the island of Borneo: *B. denigratus* T. Majewski & K. Sugiy. on *Lispinus coarcticollis* from Sabah (Malaysia), *B. fastigiatus* (Thaxt.) I.I. Tav. on *Cercyon* sp. from Sarawak (Malaysia), and *B. lispini* on *Lispinus* spp. from Sarawak and Sabah (Malaysia) (Thaxter 1931, Majewski & Sugiyama 1986). Our specimens undoubtedly represent *B. lispini*, based on (1) no blackening of the basal parts of the receptacle, (2) receptacle consisting of superposed, flattened cells, usually undivided (a few cells may be divided by two vertical septa), and (3) a subconical and rather short perithecium. One

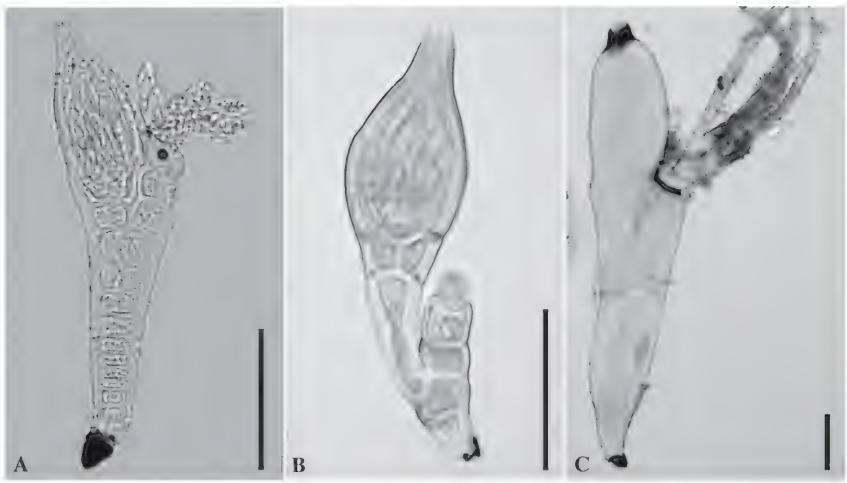

Plate 1. A. ***Blasticomyces lispini*** (FH 00313486). B. ***Cantharomyces orientalis*** (FH 00313465). C. ***Laboulbenia anoplogenii*** (FH 00313461). Scale bars = 50 μm.

studied thallus differs from the original description of *B. lispini* in displaying shallow blackening of the outer margins of receptacle basal cells 2–4.

Some *Lispinus* species have been transferred to *Nacaeus*. *Nacaeus* (*Lispinus*) *impressicollis* is a cosmopolitan species; it is broadly distributed in Australia, Japan (Naomi 1997), Central and South America, many Caribbean islands, Atlantic islands, and the Indo-Pacific biogeographic region (Irmler 2003).

Cantharomyces orientalis Speg., Anales Mus. Nac. Hist. Nat. Buenos Aires 27: 43 (1915) Plate 1B

Specimens examined: **THE PHILIPPINES, Luzon Island**, Metropolitan Manila, Manila, on "aleocharid" [sic] (*Staphylinidae*, *Aleocharinae*), Dec 1911, no collector, Thaxter 2421, slide FH 00313465 (2 thalli).

Previous records: On *Carpelimus* (syn. *Trogophloeus*) and related genera (*Staphylinidae*, *Oxytelinae*); two records were collected from *Bledius* (*Staphylinidae*, *Oxytelinae*). *Cantharomyces orientalis* is reported from Finland, Russia (Huldén 1983), Germany (Scheloske 1969), Italy (Spegazzini 1915), Poland (Siemaszko & Siemaszko 1928, 1932, Majewski 1994), the Netherlands [as *C. thaxteri*] (Middelhoek 1949), Spain (Santamaría 1989), Sweden, Switzerland (Huldén 1985), Belgium (De Kesel & Haghebaert 1991), Great Britain (Weir & Beakes 1993), Greece (Castaldo et al. 2004), Czech Republic, Slovakia (Rossi et al. 2010), and Algeria [as *C. abbreviatus*] (Maire 1920).

All 29 *Cantharomyces* species are characterized by a receptacle consisting of three superposed cells and a compound antheridium subtending a simple or variably branched primary appendage, and a perithecium having four to five

cells in each vertical row of outer wall cells (Haelewaters & De Kesel 2013). Hosts are mainly *Staphylinidae* (subfamily *Oxytelinae*, tribes *Blediini*, *Euphaniini*, and *Oxyteliini*; subfamily *Aleocharinae*, tribes *Aleocharini*, and *Oxypodini*), next to *Dryopidae*, *Limnichidae*, and *Hydrophilidae*.

Cantharomyces orientalis is a very variable species, leading to confusion among all *Cantharomyces* species occurring on *Carpelimus* s.l. (see Santamaría 2003). The Philippine thalli have the primary appendage broken above the suprabasal cell (see Majewski 1990) but otherwise correspond to the description for *C. orientalis* with cell I hyaline and contrasting with the yellowish amber toned cells II (isodiametric to trapezoidal) and III (subequal to cell II but less high), the squarish basal cell of the primary appendage with the lateral antheridium, cell VI hyaline and elongated (≤40 µm), and the perithecium 68 × 37 µm, symmetrical, and broadest near basal third.

The Philippine finding of *C. orientalis* represents the first record of this species for Asia.

Dimeromyces rugosus Thaxt., Proc. Amer. Acad. Arts 55: 245 (1920) PLATE 3A

SPECIMENS EXAMINED: **INDONESIA**, **SUMATRA**, WEST SUMATRA PROV., Mount Singgalang, on *Leiochrodes* sp. (*Tenebrionidae*, *Diaperinae*), Nov-Dec 1925, leg. Edward Jacobson, Thaxter 3466, slide FH 00313485 (4 male + 4 female thalli from the legs).

PREVIOUS RECORDS: Known from material mentioned in the original description (Thaxter 1920), on *Leiochrodes medianus* from the Solomon Islands and on *L. minutus* from Malaysian Borneo. Afterwards recorded only in Taiwan, on *Leiochrodes* spp. (Terada 1976).

Dimeromyces Thaxt. is a large genus with 109 species (Kirk et al. 2008), parasitizing *Acarini*, *Blattodea*, *Dermaptera*, *Thysanoptera*, *Coleoptera*, and *Diptera* (Santamaría 2003). Its perithecial wall showing conspicuous transverse striae above the hyaline base easily distinguishes *D. rugosus* from other species. In addition, two tiny papillae are borne on opposite sides of the perithecial apex.

Laboulbenia anoplogenii Thaxt., Proc. Amer. Acad. Arts 35: 156 (1899) PLATE 1C

= *Laboulbenia stenolophi* Speg., Redia 10: 65 (1914)

SPECIMENS EXAMINED: **INDIA**, **WEST BENGAL STATE**, Kanchrapara, on *Clivina* sp. (*Carabidae*, *Scaritinae*), 5 Aug 1944, leg. Mont A. Cazier, D. Haelew. 305, in coll. American Museum of Natural History, slides FH 00313459 (4 thalli from right elytron), FH 00313460 (1 thallus from right metatrochanter), FH 00313461 (3 thalli from left mesocoxa + mesotrochanter), FH 00313462 (2 thalli from right elytron), and FH 00313463 (1 thallus from right mesofemur).

PREVIOUS RECORDS: Described from *Anoplogenius cyanescens* [as *A. circumcinctus*] (*Carabidae*, *Harpalinae*). In Asia, beetles in *Abacetus*, *Chlaeminus* (*Carabidae*, *Pterostichinae*), *Egadroma*, *Harpalus*, *Platynus* [as *Colpodes* in Juan & Chien 1995], and *Stenolophus* (*Carabidae*, *Harpalinae*) are known to host this parasite (Santamaría

et al. 1991, Lee et al. 2002, Shen et al. 2006). Despite some confusion (see below), *L. anoplogenii* has a global distribution but has not yet been reported from South America (Santamaría et al. 1991).

Laboulbenia anoplogenii has been reported from India only once, from *Bembidion* (*Carabidae*, *Trechinae*) (Kaur et al. 1993). The same authors also report *L. stenolophi* Speg. and *L. egens* Speg. [misidentified as *L. tachyis* Thaxt.] from *Bembidion*, thereby adding the latter as a new host genus for all three parasite species. However, we suggest that this material should be re-examined.

Santamaría (1989, 1998) considers *Laboulbenia stenolophi* a synonym of *L. anoplogenii*. Other authors discriminate two species based on morphological characters (e.g., Terada 2001, Terada et al. 2004). Terada (2001) maintains that *Anoplogenius* is the only host genus for *L. anoplogenii*, and that previous records of *L. anoplogenii* from other hosts represent misidentifications. However, we observed subdivisions of cell IV, supposedly characteristic for *L. anoplogenii* on *Anoplogenius* (sensu Terada 2001) on three Roland Thaxter slides — from *Abacetus* spp.: FH 00313469 (from Sri Lanka) and FH 00313470 (from the Bengal region) and from *Stenolophus* sp.: FH 00313471 (from Massachusetts, USA). Thaxter's slide FH 00313468 (from *Stenolophus* sp. collected in Nantes, France) shows two mature thalli without transverse septa dividing cell IV. We share Santamaría's (1989, 1998) opinion that *L. anoplogenii* and *L. stenolophi* are synonyms, taking into account some degree of variability of the subdivisions of cell IV.

The species-rich genus *Clivina* is known as a host to several species of *Laboulbeniales*: *Dixomyces clivinae* (Thaxt.) I.I. Tav., *D. pallescens* (Thaxt.) I.I. Tav., *Laboulbenia clivinalis* Thaxt., *L. schizogenii* Thaxt., *L. timurensis* T. Majewski & K. Sugiy., *Ormomyces clivinae*, and *Peyritschiella clivinae* Thaxt. *Clivina* may also be an accidental host for *L. anoplogenii* ("nebenwirt"; Scheloske 1969), since it occupies the same habitat as the parasite's typical hosts (Santamaría 1998). The first record of *L. anoplogenii* on *Clivina*, an obvious consequence of an accidental infection (S. Santamaría pers. comm.), was found in the Natural Park of El Fondo, Spain, on a single specimen of *C. ypsilon* (Balazuc et al. 1983, Santamaría 1989).

Laboulbenia cafii Thaxt., Proc. Amer. Acad. Arts 35: 162 (1899)

New record: **SINGAPORE**, **East Region**, Changi, on *Remus corallicola* (*Staphylinidae*, *Staphylininae*), no date, leg. Malcolm Cameron, D. Haelew. 303, in coll. American Museum of Natural History, slide FH 00313440 (1 thallus collected from left metafemur).

Previous records: *Laboulbenia cafii* is known from America, Europe, Asia, and Oceania (Santamaría 1998). In Asia, it has been reported from Hong Kong (Thaxter 1908), India (Balazuc collection, in Santamaría et al. 1991), and Japan (Sugiyama 1973). Hosts are species belonging to the genus *Cafius* and related genera.

Laboulbenia erotylidarum Haelew., **sp. nov.** PLATE 2A,B

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Differs from all other *Laboulbenia* species on *Erotylidae* by its blackening of the basal outermost part of the outer appendage and the posterior margin of the perithecium immersed in the receptacle for about 3/4 of its length.

TYPE: The Philippines, Mindanao Island, Zamboanga Peninsula administrative region, on "erotylid" [sic] (family *Erotylidae*), no date, no collector, Thaxter 3051 (**Holotype**, FH 00313483 (slide, 1 mature thallus); **isotype**, FH 00313484 (slide, 1 mature thallus collected from the elytron)).

ETYMOLOGY: Named after the host family.

THALLUS 212–265 µm long from foot to perithecial tip, pale greyish-brown in color with only the distal 3/4 of cell I and perithecium darker. CELLS I, II, III, and IV of similar length, 2–2.5× longer than broad. Cell II with subparallel margins. CELL V wedge-shaped, slightly protruding between the perithecium and the insertion cell; located in the inner-upper corner of cell IV, which is about twice as long as cell V. Septum IV–V oblique, strongly curved. INSERTION CELL oblique, very dark, marking a strong constriction on both sides above cells IV and V, located at the distal quarter of the perithecium but separated from it. OUTER APPENDAGE consisting of up to four hyaline branches of gradually more elongate cells, resulting from up to two successive dichotomies, the first of which occurring above the large, irregular basal cell; the basal cells, the outermost suprabasal, and the following cells of the outermost branch are externally blackened. INNER APPENDAGE consisting of a basal cell that is broader than long, carrying two suprabasal cells, the inner of which gives rise to a single branch, the outer one rounded, large, giving rise to two simple branches; all branches composed of gradually more elongate cells. ANTHERIDIA not observed. Longest outer appendage 205 µm, longest inner appendage 220 µm. CELL VI trapezoidal, 1.5× longer than broad. PERITHECIUM 111–118 × 38–42 µm, fusiform, hardly inflated; the apex asymmetrical, with very prominent and rounded posterior lips, each of which bearing a conspicuous, rounded papilla; pre-ostiolar spots black, sub-opaque, both more or less merging by a pre-apical shading; the ostiole hyaline. ASCOSPORES 64–72 × 4.7–5.6 µm.

Six species of *Laboulbenia* are previously reported from *Erotylidae*: *L. parvula* Thaxt. (but see discussion below); *L. scaphidomorphi* Speg. on *Scaphidomorphus bosci* from Panama (type), Bolivia, Brazil, Ecuador, Paraguay, and Peru (Spegazzini 1915, Barragán et al. 2013); *L. nesitidis* Balazuc on *Nesitis sexnotata* from Peninsular Malaysia (Balazuc 1975); *L. encaustis* K. Sugiy. & T. Majewski on *Encaustis praenobilis* from Ecuador (Sugiyama & Majewski 1987); *L. skelleyi* W. Rossi & Bergonzo on *Pselaphacus* spp. from Brazil, Costa Rica, and Ecuador (Rossi & Bergonzo 2008; Barragán et al. 2013); and *L. mycotreti* W. Rossi on *Mycotretus* spp. from Ecuador (Rossi 2011).

Plate 2. ***Laboulbenia erotylidarum.*** A. Habitus (isotype, FH 00313484). B. Detail of inner appendage with three (1–3) branches and outer appendage with four (a–d) branches (holotype, FH 00313483). ***Laboulbenia poplitea.*** C. Habitus, with the outer appendage more than doubling the full thallus length (holotype, FH 00313480). D. Detail of the perithecial apex, with the focus on one papilla (arrow). E. Detail of the single antheridium (arrow) (isotype, FH 00313481). Scale bars: A, C = 100 μm; B = 50 μm; D, E = 25 μm.

Unlike *L. erotylidarum,* both *L. scaphidomorphi* and *L. encaustis* have the insertion cell joined to the posterior margin of the perithecium (Spegazzini 1915, Sugiyama & Majewski 1987). *Laboulbenia mycotreti* differs from the new species by its stout habitus, the unbranched outer and inner appendages, and the exceptional position of cell VI (Rossi 2011).

Laboulbenia erotylidarum, *L. nesitidis*, and *L. skelleyi* share having cell V protrude between the perithecium and the insertion cell. *Laboulbenia skelleyi*, however, differs in its unbranched outer appendage, a bifurcate inner appendage, and a much shorter and stouter general appearance (Rossi & Bergonzo 2008). *Laboulbenia nesitidis* is distinguished by a perithecium that is free for more than half of its length, a different branching pattern, and a different pigmentation of the appendages (Balazuc 1975).

Colla (1926) reported *L. parvula* also from *Erotylidae* but provided no description or illustration. We agree with Rossi & Bergonzo (2008) that the record of *L. parvula* on *Brachysphaenus bimaculatus* (Colla 1926) is doubtful. This parasite was described and is known from *Carabidae* (subfamilies *Harpalinae* and *Trechinae*). *Laboulbenia parvula* obviously differs from *L. erotylidarum* in its largely free perithecium, a cell VI that is broader than long, and the differently structured appendages, the outermost with more or less darkened lower cells (mainly at their posterior margins).

We retain the species name written on the slide labels by Roland Thaxter.

Laboulbenia poplitea Haelew., **sp. nov.** PLATE 2C–E

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Differs from other *Laboulbenia* species by its kinked cell II posterior margin, its perithecium covering most of cell III, and cells III and IV posterior margins parallel to the perithecial anterior margin.

TYPE: The Philippines, Luzon Island, Metropolitan Manila, Manila, on *Craspedophorus* sp. (*Carabidae*, *Harpalinae*, *Panagaeini*), Jan 1912, no collector, Thaxter 2549 (**Holotype**, FH 00313480 (slide, 4 mature thalli; **isotype**, FH 00313481 (slide, 3 mature thalli)).

ETYMOLOGY: From the Latin noun *popliteus* = back of the knee joint, referring to the angulated posterior margin of cell II.

THALLUS 195–212 μm long from foot to perithecial tip, light brown in color with darker cell VI and perithecium. CELLS I and II forming a pedicel; the former rectangular, 44 × 20 μm; the latter abruptly broadening upwards, distally up to 41 μm wide, its posterior margin kinked and longer (38–52 μm) than the (straight) anterior margin (30–44 μm). CELL III rectangular, 47–56 μm long, hardly to distinguish from the body of the perithecium. CELL V wedge-shaped, distal half separated from perithecial wall, located in inner-upper corner of CELL IV, which is elongated, 39–44 μm long, on average 2.4× longer than cell V. INSERTION CELL dark and thick, marking a constriction above cells IV and V, located at height of preostiolar spots of perithecium, but separated from it. OUTER APPENDAGE simple, long and slender, up to 320 μm in length, consisting of a rectangular basal cell of about 19 μm long, followed by one to three cells of similar length; the other cells forming the appendage more elongate. INNER APPENDAGE consisting of a small, subtriangular basal cell, reaching only 2/3 of the length of the basal cell of the outer appendage and bearing distally one

elongate cell that produces a slender antheridium. With age, the antheridium degenerates and elongates as a simple evanescent branch. Cell VI broader than long, wedge-shaped, its anterior side measuring 14–17 µm. Perithecium 95–106 × 37–42 µm, asymmetrical, the anterior margin nearly straight, the posterior margin strongly convex, broadest at 1/3, tapering upwards; the apex asymmetrical, ending in three rounded lips, the anterior being distinctly shorter; papillae conspicuous on anterior and posterior lips; the ostiole hyaline, very contrasting to the pre-ostiolar spots, the anterior of which is more reduced to a longitudinal stria. Ascospores 51–63 × 3.2–4.7 µm.

Laboulbenia poplitea is recognized by the following characteristics: 1) the kinked posterior margin of cell II, giving it the look of the back of the knee joint, 2) cell III covered by the body of the perithecium, and 3) the posterior margins of cells III and IV parallel to the anterior perithecial margin. In addition, the outer appendage is unbranched and can become very long, contributing to a total thallus length (from foot to tip of the outer appendage) up to 0.5 mm. In many *Laboulbenia* species damaged appendages are known to regenerate atypically. Yet, among all examined specimens of *L. poplitea*, we found no thalli with abnormal regeneration; two thalli with a normally regenerated — unbranched — outer appendage were observed.

Laboulbenia poplitea bears a superficial resemblance to *L. erecta* Thaxt., parasitic on *Platynus* spp. [as *Colpodes*] (*Carabidae*, *Harpalinae*, *Platynini*) from Mexico, which, however, has a straight cell II, an inner appendage with the suprabasal cell producing two antheridia, and an outer appendage always branched above the suprabasal cell and with sometimes an additional branch arising from the basal cell anterior side (Thaxter 1899, Haelewaters, pers. obs.). In addition and in contrast to *L. poplitea*, the perithecium of *L. erecta* does not cover cell III but has an almost symmetrical profile.

Craspedophorus is a senior synonym of *Brachyonychus*, which Roland Thaxter wrote [misspelled as "*Brachionychus*"] on the slide labels. *Craspedophorus,* an Old World genus, is very speciose. Only three species of *Laboulbeniales* have been reported on this host genus: *Laboulbenia brachyonychi* Thaxt., *L. proliferans* Thaxt., and *L. taiwaniana* Terada et al.

Laboulbenia proliferans parasitizes carabid beetles representing several subfamilies and tribes (e.g., *Brachinini*, *Licinini*, *Callistini*, *Panagaeini*) from Europe, Asia, Africa, and Oceania (Arndt & Santamaría 2004) but differs from *L. poplitea* in many respects, e.g. by the proliferation of cell V (Thaxter 1893). Also *L. brachyonychi* (Thaxter 1899) is very different morphologically, with a wholly free slender perithecium, the androstichum (cells III, IV, and V) forming a dark pigmented stalk separated from the perithecium, and both the outer and inner appendage simple and elongate (the inner one may be branched

Plate 3. A. ***Dimeromyces rugosus*** (FH 00313485), mature male thallus (m) and female thallus (f), showing transverse striae at the perithecial wall (area between dashed lines) and two papillae at opposite sides of the perithecial apex (arrow). B. ***Monoicomyces stenusae*** (FH 2535). C. ***Laboulbenia satanas*** (FH 00313478). D. ***Laboulbenia timurensis***, arrow showing the non-pigmented, lens-shaped insertion cell, above which occur subdivisions of the appendage basal cells (FH 00313449). E. ***Ormomyces clivinae***, showing ascospores oozing out of the perithecium two by two (FH 00313445). Scale bars: A = 50 μm; B–E = 100 μm.

one or twice). More recently, Terada et al. (2008) described *L. taiwaniana* on *Craspedophorus formosanus* from Taiwan, which is distinguished by the black septum between the basal and suprabasal cell of the inner appendage, next to the elongate perithecium and cell V subequal in length to cell IV.

Laboulbenia satanas Balazuc, Rev. Mycol. 37: 261 (1973) PLATE 3C

SPECIMENS EXAMINED: **THE PHILIPPINES, LUZON ISLAND,** LAGUNA PROV., Mount Makiling, on "gyrinid beetle" (*Gyrinidae*), no date, leg. Charles F. Baker, Thaxter 3244, slides FH 00313475 (12 thalli), FH 00313476 (4 thalli), FH 00313477 (26 thalli), FH 00313478 (11 thalli), and FH 00313479 (14 thalli).

PREVIOUS RECORD: The only previous report is from the Philippines (Balazuc 1973), on *Orectochilus discus* (*Gyrinidae*).

Some new *Laboulbenia* species that Thaxter intended to characterize in the never-finished sixth volume of his monumental monograph have now been described by other scientists (Haelewaters & Rossi 2014). *Laboulbenia satanas* parasitizing *Gyrinidae* is one. Thaxter had provisionally named "his" new species *L. "auriculata,"* but Balazuc (1973) formally described the same species as *L. satanas*.

The combination of cell V forming an elongated lobe along the posterior margin of the perithecium and the horn-like outgrowths at the perithecial apex is unlike any other species in the genus. *Laboulbenia bicornis* Thaxt. shares the slender (olive-)brown horn-like processes, yet these processes are differently shaped in both species (see Thaxter 1908, Plate LXVII, Figs. 1–2). In addition, *L. bicornis* is much more slender and elongated (780–950 µm) than *L. satanas* (250–390 µm). *Laboulbenia fallax* Thaxt. (on *Gyretes* spp.) and *L. rotundata* Thaxt. (on *Dineutes spinosus*) also parasitize members of the *Gyrinidae*. Both species share with *L. satanas* the unusual development of cell V, but lack any differentiation at the perithecial tip (except for two minute tooth-like projections in *L. rotundata*).

Laboulbenia timurensis T. Majewski & K. Sugiy., Trans. Mycol. Soc. Japan 27: 436 (1987) PLATE 3D

SPECIMENS EXAMINED: **PAPUA NEW GUINEA, MILNE BAY PROV.,** Modeway Bay, on *Clivina inopaca* (*Carabidae, Scaritinae*), 2 Feb 1956, leg. Leonard J. Brass, det. Philip J. Darlington, D. Haelew. 308, in coll. American Museum of Natural History, slides FH 00313447 (4 thalli from left elytron), FH 00313448 (1 thallus from right mesotibia), FH 00313449 (3 thalli from left metatibia), FH 00313450 (6 thalli from legs), FH 00313451 (1 thallus from left mesotibia), FH 00313452 (2 thalli from right elytron), and FH 00313453 (4 thalli from right elytron).

PREVIOUS RECORDS: Described on *Clivina* sp. (*epippiata* group) from Indonesia (Borneo); afterwards recorded only once, on *Clivina yanoi* from Taiwan (Majewski & Sugiyama 1986, Terada et al. 2004).

The studied thalli correspond morphologically with the figures of *L. timurensis* in Terada et al. (2004). We agree with their suggestion that "the division [into a mass of small cells] seems to be in the appendage above the insertion cell," which is undivided and lens-shaped. The parasites were found on both the legs and elytra of the host.

Monoicomyces stenusae Thaxt., Proc. Amer. Acad. Arts 51: 30 (1915) PLATE 3B

EXAMINED SPECIMEN: **THE PHILIPPINES, LUZON ISLAND,** METROPOLITAN MANILA, Manila, on *Silusa* sp. (*Carabidae*, *Scaritinae*), Dec 1911, no collector, Thaxter 2417, slide FH 00313466 (1 thallus collected from the elytron).

PREVIOUS RECORDS: Described from *Neosilusa ceylonica* [as *Stenusa* in Thaxter 1915; as *Silusa* in Thaxter 1931] (*Staphylinidae*, *Aleocharinae*) from Indonesia (Java). Afterwards reported only once, on "*Silusa kamerunensis*" from Cameroon (Thaxter 1915, 1931).

Majewski (1988) found *Monoicomyces plagiusae* Thaxt. in Japan and suggested that *M. plagiusae* and *M. stenusae* are synonyms. Before Majewski (1988), *Monoicomyces plagiusae* was known only from the type locality in Indonesia (Sumatra) (Thaxter 1931).

A single thallus mounted by Thaxter was studied. The available specimen corresponds most to the description for *Monoicomyces stenusae*. The two secondary axes on either side of the suprabasal cell are proliferated, consisting of three cells, the latter of which gives rise to two antheridia and a perithecium. Thaxter's (1915) original description mentions that the third cell is "usually terminated by two antheridia." Also, our measurements differ significantly from Thaxter's measurements of thalli from Java (Indonesia) and Cameroon; especially the cells VI (up to 114 × 32 μm) and the perithecia (144–156 × 41–47 μm) are considerably larger than reported by Thaxter himself.

Considering Thaxter's (1915, 1931) suggestion of prominent morphological variation within this species, we think it is safe to identify the Philippine thallus as *M. stenusae*, until more material becomes available. Moreover, this thallus might represent another form, restricted to the elytra, whereas Thaxter (1915, 1931) mentions only smaller, compact forms taken from the legs and "more developed" ones from the abdomen.

Ormomyces clivinae (Thaxt.) I.I. Tav., Mycol. Memoir 9: 266 (1985) PLATE 3E

NEW RECORD FROM INDIA: **INDIA, WEST BENGAL STATE**, Kanchrapara, on *Clivina* sp. (*Carabidae*, *Scaritinae*), 9 Jul 1944, leg. Mont A. Cazier, D. Haelew. 306, in coll. American Museum of Natural History, slides FH 00313443 (2 thalli from right elytron), FH 00313444 (2 thalli from distal tip abdomen), and FH 00313445 (4 thalli from right elytron).

PREVIOUS RECORDS: Recorded on *Clivina* spp. from Indonesia (type), Madagascar, Sierra Leone, and Cameroon (Balazuc 1982, Rossi 1982, Tavares 1985).

Ormomyces I.I. Tav. was erected to accommodate a single species, *O. clivinae*, which Thaxter (1915) had earlier placed in *Misgomyces*. The species is related to *Ecteinomyces trichopterophilus* Thaxt., which shares the uniseriate receptacle. However, *E. trichopterophilus* differs from *O. clivinae* in its cell III having a corner cell (which may give rise to a secondary branch), a perithecium with long

narrow neck, and in having different hosts (*Ptiliidae*, *Acrotrichinae*: *Acrotrichis* spp., *Baeocrara variolosa*) (Tavares 1985, Haelewaters et al. 2014b). Notable is that *E. trichopterophilus* has been collected in North and South America and Europe (Haelewaters et al. 2014b), while *O. clivinae* has so far been recorded only in southeastern Asia and Africa. Future collections will elucidate whether or not the geographical distributions of the two species overlap.

A thorough literature review of all *Laboulbeniales* reported from India yielded only 32 species (Thaxter 1896, 1899, 1900, 1901, 1902, 1908, 1915, 1926; Batra 1963; Kaur et al. 1993; Kaur & Mukerji 1995, 1996a,b; Pathak & Mukerji 1997; present paper). However, since India is considered one of the 17 "megadiverse" countries in the world, with the Western Ghats mountain range recognized as a biodiversity hotspot (Myers et al. 2000), we anticipate that many more species of *Laboulbeniales* will be found in this country.

Peyritschiella princeps (Thaxt.) I.I. Tav., Mycol. Memoir 9: 270 (1985)

Specimens examined: **INDONESIA**, **Lombok**, West Nusa Tenggara Prov., Sapit village, on *Philonthus tardus* (*Staphylinidae*, *Staphylininae*), Apr 1896, leg. Hans Fruhstorfer, D. Haelew. 304, in coll. American Museum of Natural History, slides FH 00313441 (1 thallus from pronotum) and FH 00313442 (1 thallus from left-hand side tergite).

Previous records: Described as *Dichomyces princeps* on *Philonthus sordidus* (*Staphylinidae*, *Staphylininae*), Massachusetts, U.S.A. *Peyritschiella princeps* is known from all continents except Antarctica, attacking beetles in the genera *Quediomacrus*, *Spatulonthus*, and *Philonthus* (Santamaría et al. 1991).

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Dictydiaethalium dictyosporangium sp. nov. from China

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Abstract — *Dictydiaethalium dictyosporangium*, described as new, is characterized by a branched pseudocapillitium and spores, which are 10–12 µm in diam. and ornamented with long ridges that sometimes form an incomplete banded reticulum. The new species, which was collected on the bark of a dead log in Henan Province, is described and illustrated by light and scanning electron micrographs. A key to three known *Dictydiaethalium* species is also provided. The holotype is deposited in the Herbarium of Mycological Institute of Jilin Agricultural University (HMJAU) in Changchun, China.

Key words — myxomycetes, taxonomy, *Dictydiaethaliaceae*

Introduction

Dictydiaethalium was established by Rostafiński in 1873. Two species are currently recognised in the genus (Kirk et al. 2008), of which only one has been reported from China (Li & Li 1989). A specimen found on the bark of a dead log in Shiziping village, Sanmenxia city, Henan province, China, in September 2013 differed from the two named species and is described here as new.

Materials & methods

Fruiting bodies and microscopic structures were examined by light and scanning electron microscopes (Zhang & Li 2013). Permanent slides were prepared according to Robbrecht (1974) by spreading a capillitium in a drop of 94% alcohol, determining color after one minute, and then mounting in Hoyer's medium (Martin & Alexopoulos 1969). Color terms are given according to Flora of British Fungi (Royal Botanic Garden Edinburgh 1969). Morphological observations and measurements were made using a stereomicroscope (20×) and optical microscope (400× and 1000×). About ten pseudoaethalia, pseudocapillitia, and 20 spores were measured using a Leica DM100 microscope, and photographs were taken with a Canon G15 camera. For SEM images, pseudoaethalia attached to a holder were coated with gold using a Hitachi E-1010 sputter

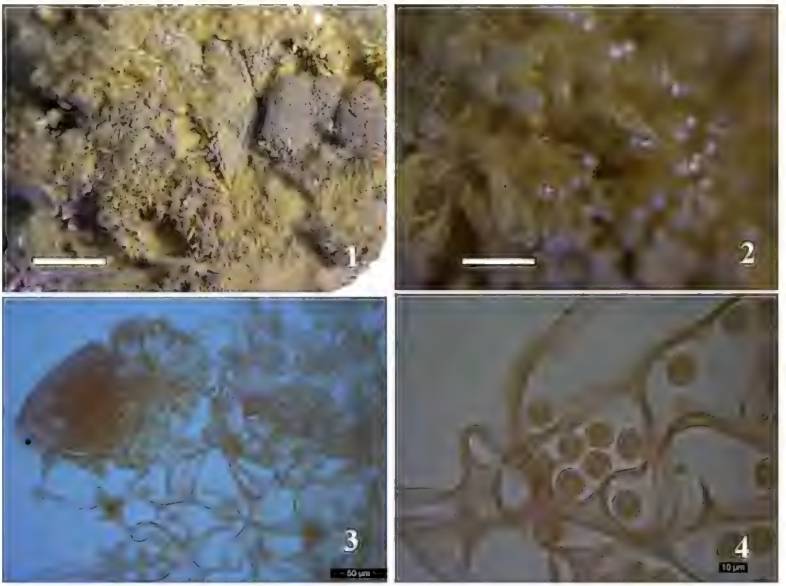

Plate 1. *Dictydiaethalium dictyosporangium* (Isotype, HMJAU10256): 1–2. Fruiting bodies; 3. Pseudocapillitium and spores (400×); 4. Pseudocapillitium and spores (1000×). Scale bars: 1 = 0.5 mm; 2 = 0.2 mm.

coating unit and examined with a Hitachi S-4800 scanning electron microscope at 10 kV at Changchun Institute of Applied Chemistry, Chinese Academy of Sciences. The specimens are deposited in the Herbarium of Mycological Institute, Jilin Agricultural University, Changchun, China (HMJAU).

Taxonomy

Dictydiaethalium dictyosporangium B. Zhang & Yu Li, **sp. nov.** Plates 1–2

MycoBank MB807341

Differs from *Dictydiaethalium dictyosporum* by its branched pseudocapillitia and from *D. plumbeum* by its spores with long ridges sometimes forming an incomplete banded reticulum.

Type—China, Henan province, Sanmenxia city, Shiziping village, on the bark surface of a dead log, 22 September 2013, Zhang Bo 100802 (**Holotype**, HMJAU10244; **isotype**, HMJAU10256).

Etymology—*dictyosporangium* (Latin) = with a branched pseudocapillitium.

Sporophores a pseudoaethalium, at maturity simulating an aethalium, effused, depressed, spreading over 22 mm, olivaceous to gray olivaceous, pulvinate, irregular in outline, more or less circular, extending up to 2.2 cm, up to 1.1 cm thick. Hypothallus shining, membranous, abundantly developed

PLATE 2. *Dictydiaethalium dictyosporangium* (Isotype, HMJAU10256), scanning electron micrographs: 1–2. Partial pseudocapillitium and spores; 3. Branched pseudocapillitium; 4. Spores ornamented with long ridges, arranged in an incomplete banded reticulum.

and surrounding the pseudoaethalium. PERIDIUM single, membranous, translucent, slender and evanescent at the base, olivaceous brown in transmitted light, smooth, persistent. COLUMELLA absent. CAPILLITIUM absent. PSEUDOCAPILLITIUM filiform, flat, thick on one side, 3–5 μm wide, smooth expect for the thickened part which bears a row of warts, branched and anastomosed, running down to the base of pseudoaethalia, yellowish green to olivaceous green, pale yellow by transmitted light. SPORES free, bright yellowish green in mass, pale yellow to colourless by transmitted light, 10–12 μm diam., ornamented with long ridges sometimes forming an incomplete banded reticulum

COMMENTS: *Dictydiaethalium* was previously reported with two species: *D. dictyosporum* Nann.-Bremek. (Nannenga-Bremekamp 1966) and *D. plumbeum* (Schumach.) Rostaf. (Lister 1894). *Dictydiaethalium dictyosporum* has minutely banded-reticulate spores (ca. 9–12 μm diam.) and unbranched and thicker (ca. 8 μm wide) pseudocapillitia, while *D. plumbeum* is diagnosed by spinulose or warted spores (ca. 8–12 μm diam.) and narrower (ca. 2 μm wide) pseudocapillitia.

Key to species of *Dictydiaethalium*

1. Spores reticulate, completely or incompletely banded 2
1. Spores spinulose or warted, not reticulate ..... *D. plumbeum*
2. Pseudocapillitia connected by threads ..... *D. dictyosporangium*
2. Pseudocapillitia not connected ..... *D. dictyosporum*

Acknowledgments

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Lophodermium quadrisporum sp. nov. (*Rhytismataceae*) on *Rhododendron faberi* subsp. *prattii*

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Abstract — *Lophodermium quadrisporum*, a new fungus found on fallen leaves of *Rhododendron faberi* subsp. *prattii* from Sichuan Province, China, is described, illustrated, and discussed. This taxon is distinguished from its closest relatives by the occurrence of both 4- and 8-spored asci. The type specimen is deposited in the Reference Collection of Forest Fungi of Anhui Agricultural University, China (AAUF).

Key words — foliicolous fungus, morphological character, taxonomy

Introduction

Lophodermium Chevall. is the largest genus of *Rhytismataceae* (*Rhytismatales*, *Leotiomycetes*, *Ascomycota;* Kirk et al. 2008) and is distributed worldwide (Cannon & Minter 1986, Lin et al. 2012). IndexFungorum (2014) lists 342 epithets for the genus, among which SpeciesFungorum (2014) accepts 172 *Lophodermium* species.

Here, a fungus on an ericaceous plant from Sichuan Province is described and designated as a new species of *Lophodermium*.

Materials & methods

Material with mature ascomata was selected from the specimen eventually designated as the holotype and described macroscopically under the dissecting microscope at 10–50× magnification. After rehydration in water for ca 10 min, the fruitbodies were sliced into 8–20 µm thick sections with a freezing microtome (YD-202, China). Microscopic features were examined in water, 5% KOH, Melzer's reagent, cotton blue in water, or lactophenol-cotton blue. Sections were mounted in 0.7% (w/v) cotton blue in water to observe the outlines of ascomata in vertical section. Gelatinous sheaths surrounding

ascospores and paraphyses were observed in water or 0.1% (w/v) lactophenol-cotton blue. The color of internal structures and ascospore contents were determined in water. For each specimen, at least 30 asci, ascospores, and paraphyses were measured in 5% KOH solution. Line and point integrated illustrations of the fruitbody external shape and internal structures were prepared using the Panasoianic XSJ-2 microscope drawing device.

Taxonomy

Lophodermium quadrisporum D.D. Lu & Y.R. Lin, **sp. nov.** FIGS 1–5

MYCOBANK MB 808815

Differs from *Lophodermium rufum* by its elliptical to lunate ascomata, grey lips, branched and swollen or circinate paraphyses, and 4- and 8-spored asci.

TYPE: China, Sichuan, Ya'an, alt. ca 3550 m, on fallen leaves of *Rhododendron faberi* subsp. *prattii* (Franch.) D.F. Chamb, (*Ericaceae*), 21 June 2009, Y.G. Liu & Y.R. Lin 2765 (**Holotype**, AAUF 68873).

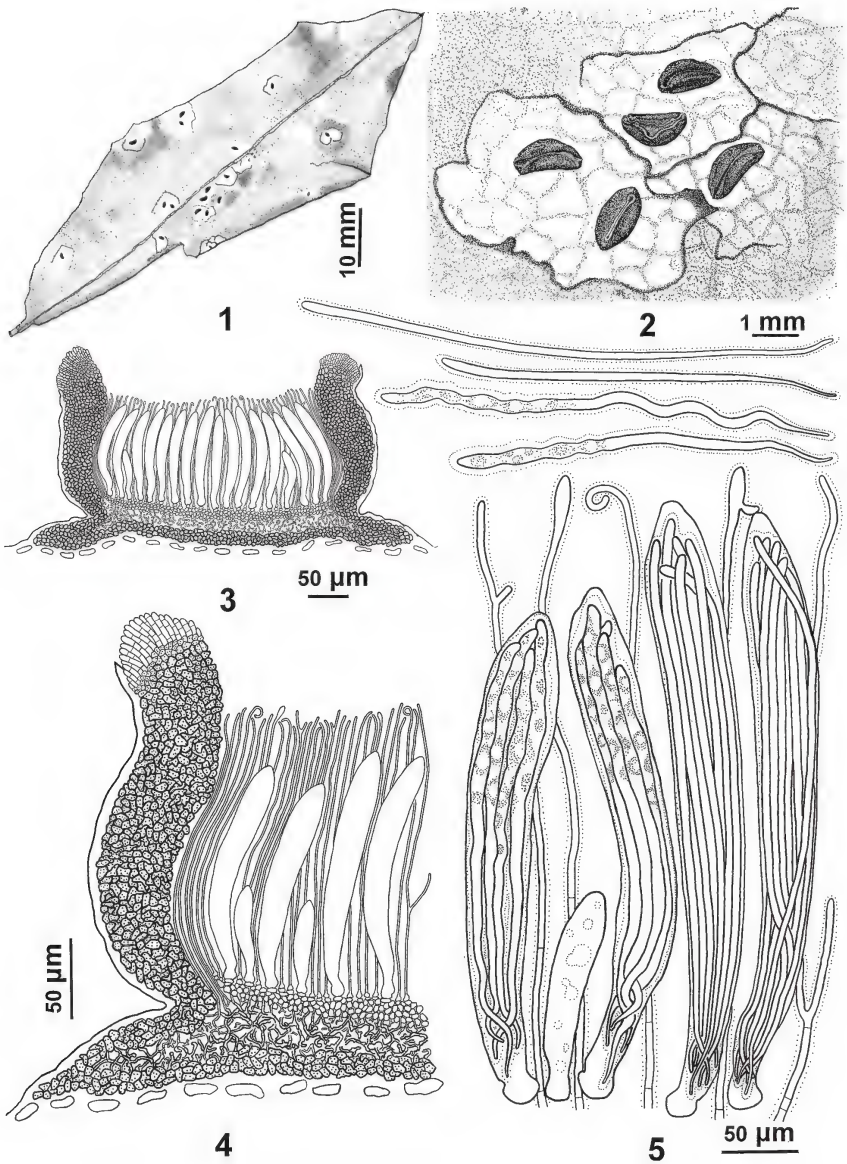
ETYMOLOGY: *quadrisporum* (Latin = 4-spored), referring to one of the two types of the asci, containing four ascospores.

COLONIES only epiphyllous, forming subround to irregular, yellow-white to grayish-yellow bleached areas 3–5 mm diam., which tend to coalesce into larger irregular shapes.

ZONE LINES frequent, dark brown to black, with various width, clearly defined or sometimes diffused, entirely or partly surrounding the paler areas.

CONIDIOMATA not observed.

ASCOMATA developing on the upper side of leaves, scattered in the bleached spots. In surface view, ascomata 680–1440 × 220–540 µm, elliptical or lunate, straight or curved to one side, black-brown to black, slightly shiny, ends rounded, obtuse or slightly acute, with a clearly marked outline, moderately rising the substratum surface, opening by a single longitudinal split nearly extend to the edge of the ascomata. Lips grey, sometimes valgus. In median vertical section, ascomata subcuticular. COVERING STROMA well developed, 45–60 µm thick near the opening, gradually thinner towards the edge, connecting to the basal stroma, mainly composed of textura angularis-epidermoidea with dark brown to black thick-walled cells 3–7 µm diam. Lip cells well developed, subhyaline, 4–7 × 2–3 µm, thin-walled, cylindrical, 0–4-septate, more or less radially arranged. BASAL STROMA black-brown, comprised of 2–4 layers of thick-walled, angular to aliform cells 3–6 µm diam. SUBHYMENIUM well developed, 18–25 µm thick, flat, consisting of colorless thin-walled textura angularis and intricata. PARAPHYSES exceeding height of asci by 20–30 µm, 1.5–1.8 µm wide, thin-walled, with septa which seem to only occur in the lower part of the paraphyses, filiform, oftenbranched, sometimes circinate or gradually swollen to 3–4 µm above, covered with a ca 1 µm thick gelatinous matrix. Asci



Figs 1–5. *Lophodermium quadrisporum* (Holotype, AAUF 68873) on *Rhododendron faberi* subsp. *prattii*. 1. Habit on a leaf. 2. Detail of ascomata and zone lines. 3. Ascoma in median vertical section. 4. Portion of ascoma in median vertical section. 5. Paraphyses, asci, and ascospores.

ripening sequentially, thin-walled, cylindrical-clavate, short-stalked, apex subacute or subtruncate-conical, without circumapical thickening, not bluing in iodine, discharging spores through a small apical hole, of two-types: ca 30% A-asci: 4-spored, 105–140 × 12–19 μm; ca 70% B-asci: 8-spored, 110–160 × 15–20 μm. 5–7-spored asci caused by spore abortion not observed. A-asci ascospores 95–125 × 3–4.5 μm, wide-filiform, often curved into a snaky shape, tapered towards the acute base, containing bright oil drops and dull-colored granules in the nodular upper half, aseptate, covered by a 0.8–2 μm thick gelatinous sheath. B-asci ascospores 100–145 × 2–2.5 μm, filiform, slight tapered to the base, arranged in a fascicle, colorless, continuous, smooth-walled, covered with a gelatinous sheath ca 0.8 μm thick.

Host species, habitat and distribution: Producing ascomata on fallen leaves of *Rhododendron faberi* subsp. *prattii*. Known only from the type locality, Ya'an, Sichuan Province, China.

Comments — *Lophodermium quadrisporum* is very similar to *L. rufum* Y.R. Lin & K. Li on *Rhododendron maculiferum* subsp. *anhweiense* (E.H. Wilson) D.F. Chamb. in the way ascomata are embedded, dehiscence mechanism, and ascal shape. However, *L. rufum* produces elliptical ascomata, reddish-brown lips, heavily carbonized tissues in outer layer of the covering stroma, nearly colorless textura angularis between the covering and basal stroma, uniformly 8-spored asci, and unbranched paraphyses (Xu et al. 2001).

Lophodermium pachychilum Y.R. Lin & Z.S. Xu is distinguished from the new species by absent zone lines, wide-elliptical intra-epidermal ascomata dehiscing by an elongate-elliptical split, a covering stroma composed of textura angularis with pale thin-walled cells near the edge, a subhymenium consisting of textura porrecta, and paraphyses agglutinated to form an epithecium above the asci (Xu et al. 2001).

In addition, *Lophodermium quadrisporum* resembles *Lophomerum ponticum* Minter on *Rhododendron ponticum* L. morphologically, but *L. ponticum* has smaller ascomata (only 600 μm long), much shorter and narrower asci (65–105 × 9–15 μm), shorter paraphyses just as long as or slightly longer than the asci, and shorter ascospores (45–80 μm) aseptate or 1–3-septate (Minter 1980).

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Diploschistes xinjiangensis, a new saxicolous lichen from northwest China

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Abstract — *Diploschistes xinjiangensis* from Xinjiang in northwest China is described as new to science. The species is characterized by the thick bluish gray thallus, asci with eight large ellipsoid ascospores, and the presence of diploschistesic and lecanoric acids. It grows on rock in the semiarid region at elevations of ca. 1700 m. ITS rDNA sequence analyses support the taxonomic distinctness of this species.

Key words — Asia, biodiversity, *Graphidaceae, Ostropales,* taxonomy

Introduction

Diploschistes Norman includes crustose lichens with a blackish pseudoparenchymatous proper exciple, lateral paraphyses, and a trebouxioid photobiont (Lumbsch 1989; Lumbsch & Mangold 2007). The genus is widely distributed in arid and semiarid regions worldwide, with approximately 35–43 species known (Pérez-Vargas et al. 2012; Fernández-Brime et al. 2013). *Diploschistes* species occur mostly on rocks, some on soil, and a few rarely on wood or bark (Lumbsch & Mangold 2007). The genus exhibits a remarkable variability in ascomatal morphology, varying from perithecioid to urceolate and lecanoroid (Lumbsch 1989; Lumbsch & Mangold 2007; Mangold et al. 2009). Despite this variation, the genus is currently regarded as monophyletic and accommodated within the *Graphidaceae* based on molecular studies (Martín et al. 2003; Frisch et al. 2006; Fernández-Brime et al. 2013).

[#] These authors contributed equally to this work.

The lichen biota of northwest China is rich, with more than 670 species and 127 genera so far reported (Abbas & Wu 1998; Guo 2005). Nevertheless, new species continue to be discovered in this region, and knowledge of its lichen diversity remains incomplete. Of the eight *Diploschistes* species recorded in China (Wei 1991), three were described from the northwest region (Guo 2005).

An additional *Diploschistes* species recently collected by the first author in Xinjiang, northwest China, is named here as *D. xinjiangensis*. We present a taxonomic account based on its morphological and chemical characters and assess the phylogenetic affinities of the new species from analyses of nrDNA ITS sequences obtained from GenBank and two samples of the type specimen.

Materials & methods

The lichen specimens were collected from South Mountain in Urumqi, Xinjiang, China, and are deposited at the Herbarium Mycologicum Academiae Sinicae-Lichenes (HMAS-L) and the Lichen Section of Botanical Herbarium, Xinjiang University (XJU). The morphology was examined using a Zeiss Stemi SV 11 stereomicroscope. For microscopical examination, sections were cut by hand using a razor blade and were mounted and observed in water. Anatomical structures and hymenial characters were studied with a Zeiss Axioskop 2 plus light microscope and photographed using a Nikon Digital Camera D50. Chemical constituents were identified by thin-layer chromatography using solvent system C as outlined by Orange et al. (2010).

DNA extraction, amplification and sequencing. Two thallus fragments with ascomata were sampled from the type specimen for DNA extraction. The DNA was extracted using the DNAsecure Plant DNA Kit (Tiangen, China) following the manufacturer's protocol. Amplification of the ITS region followed the methods described in Martín et al. (2003) with modification. The whole ITS region (ITS1, 5.8S, and ITS2) of the nrDNA repeat tandem was targeted for the Polymerase Chain Reaction using the primers ITS1 with ITS4 (White et al. 1990) directly. The amplification was performed in a 25 µL volume containing 0.75 units of TransStart Taq Polymerase (Tiangen, China), 2.5 µL of its buffer, 0.5 µL of a 5 µM solution of the primers, 2 µL of 2.5 mM for each dNTP solution, and 1 µL of genomic DNA. Thermocycling protocols: 95°C for 3 min linked to 35 cycles at 94°C for 30 s, 54°C for 30 s, and 72°C for 1 min, with a final extension of 72°C for 10 min. PCR products were screened on 1% agarose gels stained with ethidium bromide. The PCR products were sequenced by Genewiz Inc. (Beijing).

Two newly obtained sequences were submitted to GenBank (see Fig. 1 for accession numbers). The beginning and end of the ITS1 and ITS2 spacers were determined by comparison with sequences available from GenBank (e.g., AJ458286, labelled as *Diploschistes gypsaceus*, but actually *D. rampoddensis* according to Martín et al. 2003: 28). We excluded the 3′ end of the 18S gene (SSU), and the 5′ end of the 26S gene (LSU) from the analyses. Our specimen sequences were aligned with the most similar taxa represented by ITS sequences in GenBank (Fig. 1). The representative taxa were selected based on their morphological characters, the results of Blast searches of sequence data, and the literature (Martín et al. 2003; Fernández-Brime et al. 2013).

Phylogenetic analysis and sequence comparisons. The ITS sequences of our two samples and the 16 other reference sequences (including *Thelotrema lepadinum* as outgroup) were aligned both by ClustalW and Muscle in MEGA 5 (Tamura et al. 2011). The alignment matrix was realigned by StatAlign for reliable measurement of the accuracy of the results (Novák et al. 2008). The final matrix (submitted to TreeBase with accession number S15164) can be obtained from the corresponding authors.

The evolutionary history was inferred both by using the Maximum Likelihood method (ML) based on the Kimura 2-parameter model in MEGA5 and using Bayesian inference (PP) based on GTR model with rates = Invgamma. The analyses involved 18 nucleotide sequences. Absolute distances were also calculated in MEGA5, using the number of base differences between sequence pairs, with all gaps removed from each sequence pair.

Results & discussion

Phylogenetic analysis

The entire ITS region was successfully sequenced for the 2 type samples. The sequence lengths for both samples = 499 bp for the entire ITS1 + 5.8S +

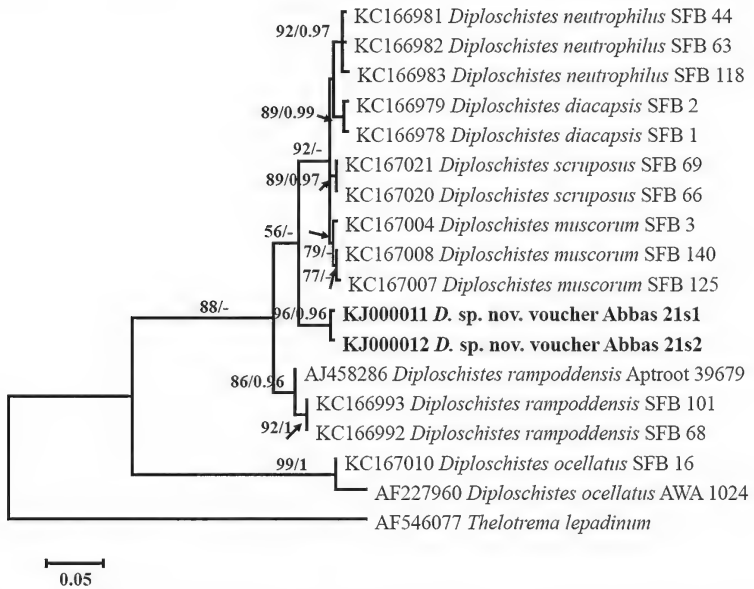

Figure 1. Phylogenetic relationships inferred from ITS sequences of *Diploschistes xinjiangensis*, *D. ocellatus*, and species in the *D. scruposus* group (with *Thelotrema lepadinum* as outgroup). Support is indicated for branches characterized by bootstrap frequencies exceeding 50% under the Maximum Likelihood method, and posterior probabilities >0.95 from Bayesian Inference. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Specimen collectors: AWA = Alan W Archer (Australia); Aptroot = André Aptroot (Netherlands); SFB = Samantha Fernández-Brime (Spain).

ITS2 region. The partial sequences containing the SSU 3′ end and LSU 5′ end are included in the data submitted to GenBank (578 bp total). Some positions (especially in the ITS2 region) in our sample sequences were difficult to align with the reference sequences and were excluded from the matrix. There were a total of 467 positions in the final dataset. All positions were used in the phylogenetic analyses.

The ITS sequences indicate that *D. xinjiangensis* probably belongs to the *D. scruposus*-group (sensu Martín et al. 2003) with close affinities to the pantropical species, *D. rampoddensis* (93% identity and 2% gap) and *D. neutrophilus* (93% identity and 3% gap). The evolutionary history was inferred as the tree with the highest log likelihood (–1582.0654). In the phylogenetic analyses, there was very strong support for the monophyly of *D. xinjiangensis* (ML = 96%; PP = 0.96) and *D. rampoddensis* (ML = 86%; PP = 0.96), but only weak support (ML = 56%) for the relationship between *D. xinjiangensis* and a sister clade including *D. diacapsis*, *D. muscorum*, *D. neutrophilus*, and *D. scruposus* (Fig. 1).

Table 1. Absolute distances for alignment sequences of ITS region (gaps ignored in pairwise comparisons) between *Diploschistes xinjiangensis* and related species in the *D. scruposus* group. Infraspecific distances are indicated with bold font.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1. *D. xinjiangensis* S1 KJ000011														
2. *D. xinjiangensis* S2 KJ000012	**2**													
3. *D. rampoddensis* KC166993	22	22												
4. *D. rampoddensis* KC166992	22	22	**0**											
5. *D. rampoddensis* AJ458286	20	20	**4**	**4**										
6. *D. neutrophilus* KC166981	18	18	25	25	22									
7. *D. neutrophilus* KC166983	18	18	25	25	22	**3**								
8. *D. neutrophilus* KC166982	18	18	25	25	22	**2**	**3**							
9. *D. diacapsis* KC166979	17	17	22	21	19	7	7	7						
10. *D. diacapsis* KC166978	17	17	24	23	21	7	7	7	**2**					
11. *D. scruposus* KC167021	15	15	22	22	19	6	6	6	7	7				
12. *D. scruposus* KC167020	15	15	22	22	19	6	6	6	7	7	**0**			
13. *D. muscorum* KC167008	16	16	22	22	19	6	6	6	7	7	4	4		
14. *D. muscorum* KC167004	16	16	22	22	19	6	6	6	7	7	4	4	**2**	
15. *D. muscorum* KC167007	17	17	23	23	20	7	7	7	8	8	5	5	**1**	**3**

Absolute distances for the aligned sequences of the ITS region also support the separation of a new species. In our sequence matrix, distances between infraspecific samples are <5, while distances between species are ≥5 (Table 1).

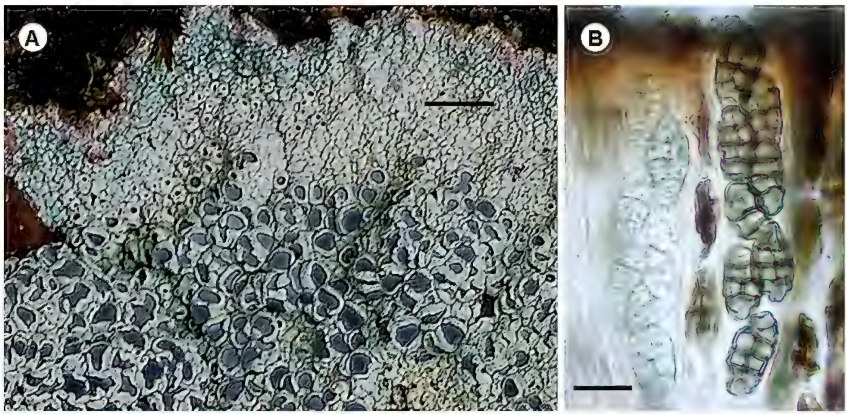

Figure 2. *Diploschistes xinjiangensis* (holotype). A: general habit; B: Asci and ascospores. Scale bars: A = 5 mm; B = 20 μm.

Taxonomy

Diploschistes xinjiangensis A. Abbas & S.Y. Guo, **sp. nov.** Figure 2

MycoBank MB 807412

Differs from *Diploschistes rampoddensis* by its thick bluish gray thallus, its 8-spored asci, and its long broad ascospores.

Type: China. Xinjiang: Urumqi Co., South Mountain, Aketa, 43°22′N 86°48′E, alt. 1750 m, 3 Aug. 2011, A. Abbas 11821 (**Holotype**, HMAS-L; **isotype**, XJU; GenBank KJ000011, KJ000012).

Etymology: The specific epithet *xinjiangensis* refers to the province where the type specimen was collected.

Thallus saxicolous, crustose, rimose-areolate, bluish grey to grayish white, thick, ≤1.5 mm thick. Upper surface dull, without pruinose. Medulla white, amyloid (I+ blue). Photobiont trebouxioid with cells ≤12 μm diam. Prothallus not visible. Vegetative propagules absent. Ascomata apothecia, exposed. Disc urceolate, without pruinose, orbicular, 0.8–2.0 mm diam. Proper exciple dark brown, 60–100 μm thick. Hymenium hyaline, 150–180 μm high, not inspersed. Hypothecium yellowish brown, 15–30 μm thick. Paraphyses 1-2 μm thick, simple, apices not thickened. Asci cylindrical, 100–130 × 20–20 μm, 8-spored. Ascospores ellipsoid, brown, muriform, with 3–5 transverse and 1–2 longitudinal septa, 24–33(–39) × 12–18 μm. Pycnidia unknown.

Spot tests —K+ yellow, C+ and KC+ red, PD–.

Secondary metabolites —Diploschistesic and lecanoric acids detected (TLC).

Ecology — *Diploschistes xinjiangensis* grows on rock. It is known only from the type locality at an elevation of 1700–1750 m in northwest China.

Additional specimens examined — **CHINA. Xinjiang:** Urumqi, South Mountain, Aketa, alt. 1700 m, 27 Aug. 2007, A. Abbas 7858, 7859 (XJU).

Comments — *Diploschistes xinjiangensis* is characterised by the thick, bluish gray thallus, the 8-spored asci, and the large ellipsoid spores. In morphology and habitat, this species is very similar to the 8-spored specimens of *D. rampoddensis*. However, *D. rampoddensis* has a thin grayish yellow thallus, shorter (18–24 µm) narrower (7–12 µm) ascospores, and a pantropical distribution and lacks diploschistesic acid, (Lumbsch 1993; Pant & Upreti 1993; Fernández-Brime et al. 2013).

Previous studies that have used DNA sequence data for species recognition in lichens required both monophyly in single-locus ITS phylogenies and diagnostic morphological differences (e.g., Han et al. 2013). Our morphological and molecular data for *D. xinjiangensis* satisfy these criteria.

Acknowledgements

The authors thank Dr. André Aptroot (ABL Herbarium, Soest, The Netherlands), Prof. Liu-Fu Han (Hebei Normal University, Shijiazhuang, China), and Prof. John A. Elix (Australian National University, Canberra, Australia) for presubmission review. S.Y. Guo was awarded grants by the National Natural Science Foundation of China (nos. 30770012, 31370067) and A. Abbas was awarded grants by the Natural Science Foundation of China (Nos. 30960003, 31150003). The valuable assistance given by colleagues is gratefully acknowledged.

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Uncispora hainanensis sp. nov. isolated from decayed leaves

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Abstract—A new species, *Uncispora hainanensis*, is described and illustrated. It is characterized by narrowly obclavate or cylindrical, slightly curved at the apical cell, mostly 3-septate, subhyaline or pale brown conidia.

Key words —fungal diversity, aquatic hyphomycetes, taxonomy

Introduction

Saprobic dematiaceous hyphomycetes are highly diverse on plant material in China, where many new genera and species have recently been discovered (Zhang et al. 2009, 2011; Ma et al. 2012a,b,c). During our ongoing survey of anamorphic fungi in Hainan Province in southwest China, one new species with morphological characteristics of *Uncispora* was collected.

Uncispora was introduced by Sinclair & Morgan-Jones (1979) based on *U. harroldiae* R.C. Sinclair & Morgan-Jones. The genus is characterized by brown, macronematous, synnematous or fasciculate conidiophores, and subhyaline to pale brown, obclavate conidia that are curved or hooked at the apex and truncate at the base. *Uncispora* is similar to *Sporidesmium* Link (Ellis, 1971) in its holoblastic, monoblastic, integrated, non-cicatrized conidiogenous cells and broadly truncate conidia but differs by its occasionally branched and synnematous conidiophores and its unique conidia with curved or hooked terminal cells. There are only two species published, *U. harroldiae* and *U. sinensis* (Yang et al. 2011). Here, we propose a third species.

[#] Jian-Ying Li & Min Qiao contributed equally to this work

Materials & methods

A culture was isolated from submerged decayed leaves in a river in the Wuzhishan National Nature Reserve. A piece of 3–4 × 6–7 cm decayed leaf was placed on the surface of CMA (20 g cornmeal, 18 g agar, 40 mg streptomycin, 30 mg ampicillin, 1000 mL distilled water) for ten days; single conidia were isolated using a sterilized toothpick while viewing with a Olympus BX51 microscope and cultivated on CMA in Petri plates. Morphological observations were made from CMA after incubation at 25°C for one week. Conidial size and septation and conidiophore size were determined by measuring more than 50 elements. Pure cultures and a permanent slide were deposited in the herbarium of the Laboratory for Conservation and Utilization of Bio-resources, Yunnan University, Kunming, Yunnan, P.R. China (YMF).

Taxonomy

Uncispora hainanensis Jian Y. Li & Z.F. Yu, **sp. nov.** PLATE 1

MYCOBANK MB 808816

Differs from *Uncispora* spp. by its slightly curved apical cell and smaller conidia.

TYPE: PR China, Hainan Province, Wuzhishan National Nature Reserve, 18°54′N 109°40′E, elev. 754 m, isolated from decayed leaves, Dec. 2011, G. Z. Yang (**Holotype**, YMF1.04038; ex-type culture, YMF1.040381).

ETYMOLOGY: *hainanensis* refers to the province in which the species was found.

COLONIES pale brown to brown, growing moderately slowly on CMA, attaining 25 mm diam. after 7 days at 25°C. Vegetative hyphae hyaline to pale brown, branched and septate smooth, commonly 1–30 µm wide, aerial mycelium sparse, hyaline, septate, and branched. CONIDIOPHORES macronematous, synnematous, or sometimes in a fascicle of a few, very rarely single, arising terminally or laterally on hyphae, erect, frequently branched, pale brown to mid brown, smooth and septate, 55–62 × 2.5–3 µm. CONIDIOGENOUS CELLS determinate, integrated, terminal, cylindrical, monoblastic, or extending percurrently to produce several terminal and subterminal conidia in fascicles. CONIDIA narrow clavate to cylindrical (19–)29–52 × 2–3 µm, tapering and curved at the apex, truncate at base, 1–5-septate, mostly 3-septate, subhyaline to pale brown, smooth, sometimes verruculose at the lower cell .

COMMENTS: *U. hainanensis* is distinguished from the other two *Uncispora* species by its conidia with a curved terminal cell. Conidia of *U. harroldiae* are larger (62–78 × 4–5 µm) with terminal cells that are mostly hooked. In *U. sinensis* the conidial apices only sometimes hook and its 0–7 septate conidia are much larger (67–89 × 2–3.5 µm). Moreover, in *U. sinensis* the conidia are much more densely arranged on the conidiophores than in *U. hainanensis*.

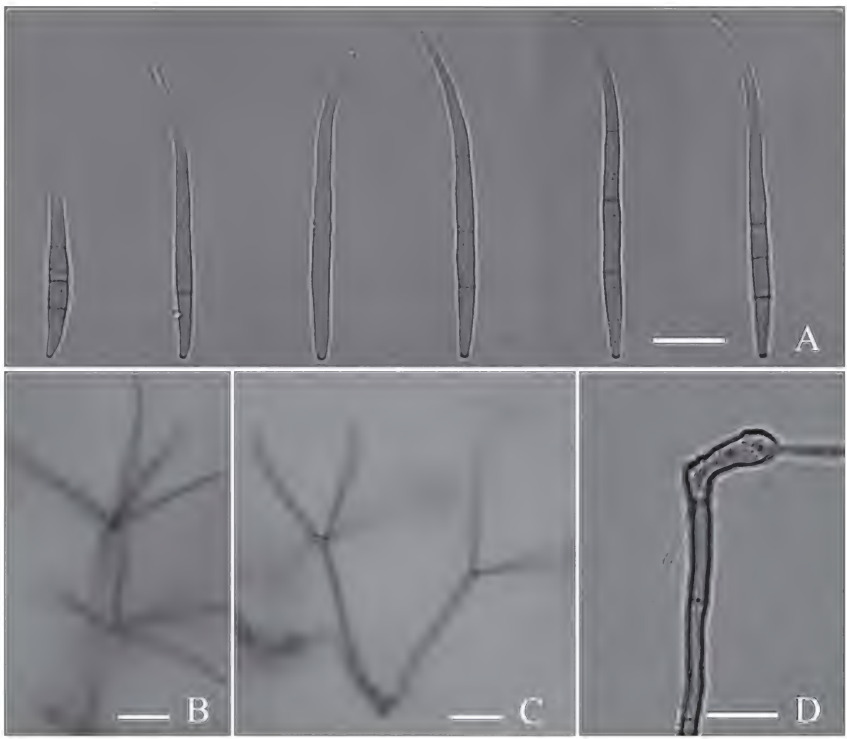

Plate 1. *Uncispora hainanensis* (holotype, YMF 1.04038). A. Conidia. B, C. Conidiophores bearing conidia in clusters. D. Conidiophore. Scale bars: A, D = 10 μm; B, C = 20 μm.

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Lactarius vesterholtii, a new species from India

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Abstract — *Lactarius vesterholtii* is proposed as new to science. It is characterized by a greyish brown dry subvelutinous pileus, very crowded light yellow lamellae turning orange when bruised, white copious latex becoming reddish orange in contact with the lamellae, a light yellow spore print, amyloid basidiospores ornamented with high ridges that forms an incomplete reticulum, the absence of pleuro- and cheilomacrocystidia, a trichopalisade to lamprotrichopalisade-type pileipellis, and a palisade to lampropalisade-type stipitipellis. The new species is compared with allied Asian and extralimital taxa. A key to the Indian taxa of *L.* subg. *Plinthogali* is also provided.

Key words — macrofungi, *Plinthogali*, *Russulaceae*, Sikkim, taxonomy

Introduction

Lactarius subg. *Plinthogali* (Burl.) Hesler & A.H. Sm., one of the well established groups amongst the common milkcaps [*Lactarius* Pers. and *Lactifluus* (Pers.) Roussel], is characterized by a pileus that is dry, velutinous to velvety, and white to buff, brownish to grey or blackish; distant to crowded lamellae; a stipe that is mostly concolorous with the pileus; context or latex in contact with the context mostly changing pinkish to reddish; a spore print that is buff, pale yellow, ochraceous, or darker but never white; basidiospores ornamented mostly with high ridges or warts either arranged in a parallel/zebroid pattern or aligned and connected in a broken to complete reticulum; macrocystidia mostly absent; and a pileipellis that is a palisade, trichopalisade, or trichoderm (Basso 1999, Das & Sharma 2004, Stubbe et al. 2008). A closely related group, *Lactifluus* subg. *Gerardii* (A.H. Sm. & Hesler) Stubbe [≡ *Lactarius* subg. *Gerardii*], can be distinguished by a white spore print and more rounded cellular elements in a palisade-type pileipellis (Stubbe et al. 2010, Verbeken & Nuytinck 2013). Ten taxa are known from *Lactarius* subg. *Plinthogali*

in India: *L. subvernalis* var. *himalayensis* Atri et al., *L. subvernalis* var. *alboochraceus* Hesler & A.H. Smith, *L. crenulatus* K. Das & Verbeken, *L. fuliginosus* (Fr.) Fr. [= *L. fumosus* Peck], *L. picinus* Fr., *L. montoyae* K. Das & J.R. Sharma, *L. lignyotellus* A.H. Sm. & Hesler, *L. croceigalus* K. Das & Verbeken, *L. lignyotus* Fr. var. *lignyotus,* and *L. lignyotus* var. *canadensis* A.H. Sm. & Hesler (Atri et al. 1993, 1994; Das & Sharma 2005; Das & Verbeken 2012).

A number of interesting ectomycorrhizal mushrooms were collected during a recent macrofungal exploration of the North district of Sikkim Himalaya. Detailed examination of the collections resulted in the discovery of a new species within *Lactarius* subg. *Plinthogali,* proposed here as *Lactarius vesterholtii.*

Materials & methods

Macromorphological features were recorded from the fresh basidiomata in the field and base camp. Macrochemical reactions with KOH, $FeSO_4$ and Guaiacol were noted. Basidiomata were dried with a field drier. Fresh and dry basidiomata were photographed using Nikon D300s, Olympus C-5060, Leica DFC550, and Nikon-DS-Ni1 cameras. Color codes and terms used are mostly from Kornerup & Wanscher (1978). Micromorphological characters were recorded with the help of compound microscope (Nikon Eclipse Ni-U) from free hand sections of dry samples mounted in a mixture of 5 % KOH and Phloxine, 30 % glycerol, and Melzer's reagent. Drawings were made with a drawing tube attached to an Olympus CX41 microscope (1000×). Basidium length excludes length of sterigmata. Basidiospore measurements and features were noted in side view from twenty basidiospores. Spore measurements and length/width ratios (Q) are presented as minimum–**mean**–maximum. Herbarium name follows Holmgren et al. (1990).

Taxonomy

Lactarius vesterholtii K. Das & D. Chakr., **sp. nov.** PLATES 1, 2

MYCOBANK MB 808854

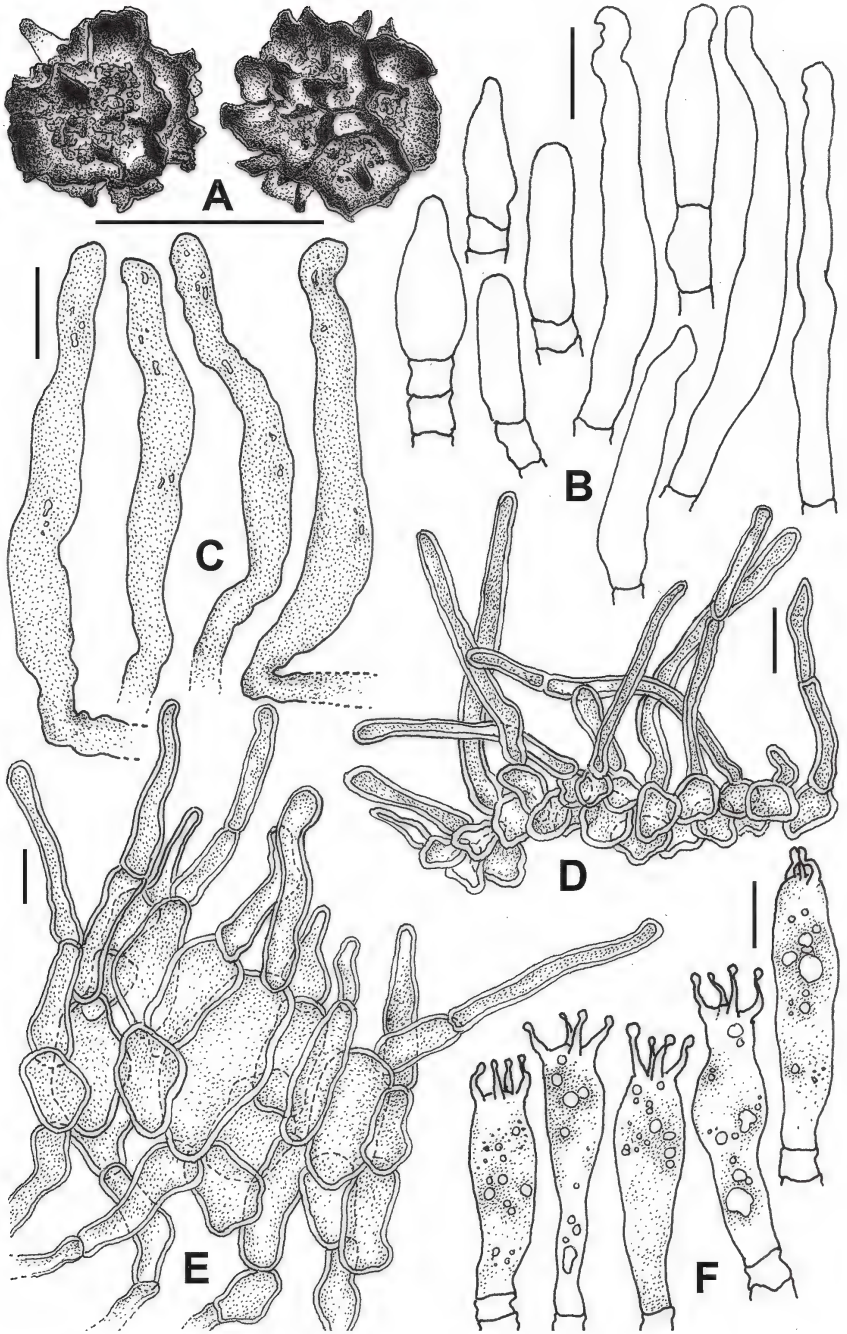
Differs from *Lactarius montoyae* by its very crowded lamellae and smaller reticulate basidiospores and from *L. croceigalus* by its very crowded light yellow lamellae that turn light orange to orange when bruised, its unbranched pleuropseudocystidia, and its lack of pileopseudocystidia.

TYPE: India. Sikkim: North District, Bansoi, 27°41′56.2″N 88°34′10.6″E, alt. 2323 m, 31.VII.2013, K. Das, KD-13-84 (**Holotype**, CAL).

ETYMOLOGY: in recognition of the late Dr. Jan Vesterholt (Denmark) for his contribution to the family *Russulaceae*

PILEUS 58–75 mm diam., convex when young, becoming planoconvex to shallowly depressed or slightly infundibuliform, never umbonate; surface rough

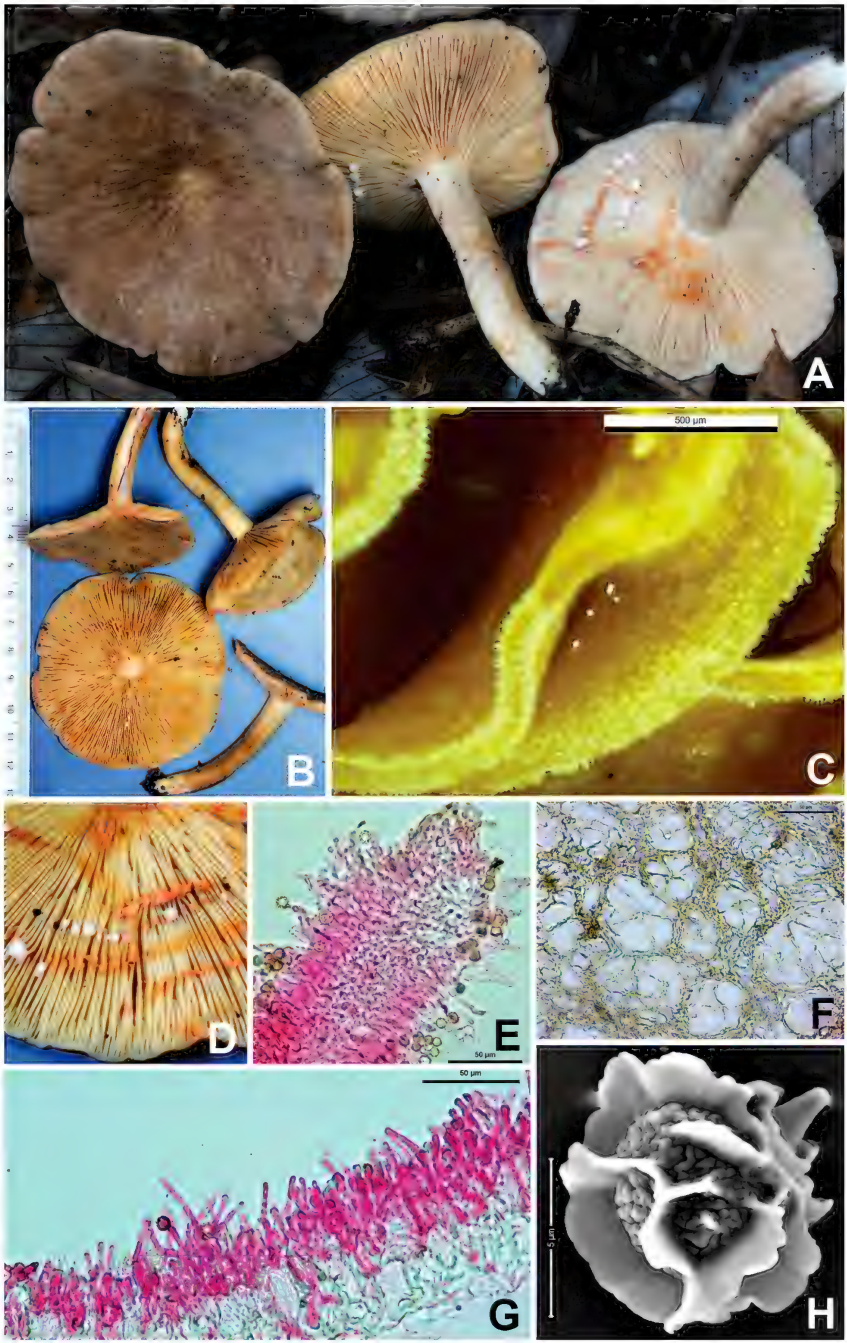
PLATE 1. *Lactarius vesterholtii* (KD 13-84, holotype). A. Basidiospores; B. Cheiloleptocystidia; C. Pleuropseudocystidia; D. Cross-section through stipitipellis; E. Cross-section through pileipellis; F. Basidia. Scale bars = 10 μm.


A
B
C
D
E
F

to subvelutinous, mostly radially wrinkled, dry to moist, never viscid, light brown (6D4) to greyish brown (6D3) or sometimes pale yellow (4A3) to greyish yellow (4B3); margin decurved to incurved, irregularly wavy or interrupted at maturity. Lamellae narrowly adnate to subdecurrent, very crowded (23/cm at pileus-margin), brittle, light yellow (4A4), turning light orange (5A5) to orange (5A7) when bruised; edge smooth but, minutely hairy (under stereo-zoom microscope), concolorous; lamellulae abundant, in 7–8 series. Stipe 55–75 × 8–13 mm, central, more or less cylindrical with tapering base; surface more or less smooth, yellowish white (4A2) at apex, towards base greyish orange (5B3) to brownish orange (5C4) or concolorous to pileus, then paler to pale yellow (4A3) near base and finally white at base, turning light orange (5A5) when bruised. Context white, becoming pale yellow (4A3), light orange (5A4) or darker when exposed, turning pale orange (5A3) with $FeSO_4$, reddish brown (8D7) with guaiacol and yellowish white (1A2) to pale yellow (1A3) with KOH. Latex abundant, white, unchanging when kept isolated, turning yellowish white with KOH but, on contact with the cut lamellae becoming pinkish white (7A2) after 3–4 minutes, then finally (or after drying) reddish orange (7A6). Odor indistinctive. Taste slightly sour initially, then acrid. Spore print light yellow (4A4).

Basidiospores 7.0–**7.5**–8.5 × 6.5–**7.1**–8.0 µm (n = 20, Q = 1.00–**1.05**–1.14), globose to subglobose; ornamentation amyloid, composed of high ridges or wings (up to 2.3 µm high) with remarkably and irregularly crenulate to lobed margin which aligned or connected to form broken to incomplete or almost complete reticulum, few high isolated subspinoid warts and isolated to jointed conical to irregular numerous minute warts (often in convoluted pattern) between high ridges; plage mostly distally amyloid. Basidia 31–53 × 8–12 µm, 4-spored, narrowly clavate to clavate, subcylindric to subclavate or ventricose; sterigmata 5–8 × 1.5–2 µm. Pleuromacrocystidia absent. Pleuropseudocystidia 4.5–7.5 µm wide, fairly common, cylindrical to subcylindrical with somewhat tortuous base, content slightly dense. Lamella-edge sterile. Cheilomacrocystidia absent. Cheiloleptocystidia 17–58 × 5–8 µm, abundant, cylindrical to subfusoid with rounded or subcapitate apex, sometimes tortuous towards apex, emergent to 35 µm, often septate, hyaline in KOH, never with brown pigmentation. Cheilopseudocystidia absent. Subhymenium layer 13–23 µm thick, pseudoparenchymatous, composed of irregular cells of 7–14.3 × 4–11 µm. Hymenophoral trama with some lactifers.

Plate 2. *Lactarius vesterholtii* (KD 13-84, holotype). A, B. Fresh basidiomata; C. Lamellar edge under stereo-zoom microscope; D. White latex turning reddish orange on cut lamellae; E. Lamellar edge, cross-section; F. Sphaerocyst nest in stipe trama; G. Pileipellis, radial section; H. Basidiospore (SEM). Scale bars: C = 0.5 mm; E–G = 50 µm; H = 5 µm.


A
B
C
500 µm
D
E
50 µm
F
G
50 µm
H
5 µm

Pileipellis 84–120 µm, two layered (supra- and subpellis), trichopalisade to lamprotrichopalisade, pileopseudocystidia absent. Suprapellis composed of thick-walled hair-like erect often septate hyphal elements; terminal cells 13–53 × 4–7.5 µm, cylindrical, ventricose, subfusoid, clavate or irregular with rounded to subcapitate apex, brown to grey pigmented, wall 0.8–1.3 µm thick. Subpellis composed of somewhat compact oval, ellipsoid to elongate or irregular thick-walled (up to 1.5 µm thick) cells with brown to grey pigmentation. Pileus trama composed nests of sphaerocytes and surrounding branched septate hyphae (4–8 µm wide). Stipitipellis up to 110 µm thick, two layered, palisade to lampropalisade. Suprapellis composed of thick-walled hair-like erect often septate hyphal elements; terminal cells 8–60 × 4–7 µm, brown-pigmented, mostly cylindrical with subfusoid to subcapitate or rounded apex. Subpellis mostly of thick-walled isodiametric brown pigmented cells. Stipe trama composed of numerous nests of sphaerocysts and hyphae.

Ecology & Distribution — Gregarious to caespitose, in temperate mixed (broad-leaf & coniferous) forest under *Lithocarpus pachyphyllus* (Kurz) Rehder. July. Rare.

Notes — The combination of features, such as the brown to greyish brown subvelutinous pileus, white latex turning pinkish white to reddish orange, globose to subglobose highly ornamented winged spores, absence of macrocystidia, and the trichopalisade pileipellis, places this species in subg. *Plinthogali*. Its very crowded lamellae, emarginate lamellar edge, sour to acrid taste, spores with combination of high wings and numerous minute warts, abundant cheiloleptocystidia, and lack of pileocystidia makes it distinct among the known taxa of this subgenus.

Lactarius montoyae (reported from India and Thailand), *L. croceigalus* (reported from India), and *L. crassiusculus* H.T. Le & Stubbe (reported from Thailand) are some Asian species that invite comparison with *L. vesterholtii*. *Lactarius montoyae* is distinguished by its distant (4/cm) lamellae, unchanging latex, ochraceous to orange-yellow spore print, and larger (7.5–10 × 7.3–9.3 µm) non-reticulate spores (Das & Sharma 2004, Le et al. 2007). *Lactarius croceigalus* can be separated by its distant (6/cm) lamellae, which are unchanging on bruising, the possession of pileopseudocystidia, and branched pleuropseudocystidia (Das & Verbeken 2012). *Lactarius crassiusculus* has close lamellae, abundant cheilomacrocystidia, a palisade pileipellis, and trichoderm-type stipitipellis (Le et al. 2007).

Lactarius acris (Bolton) Gray and *L. pterosporus* Romagn. are two superficially similar European species. However, *L. acris* is characterized by distinctly viscid to sticky pileus, latex turning coral-pink even when isolated from

lamellar tissues, and an ixotrichoderm- or ixotrichopalisade-type pileipellis; *L. pterosporus* can be separated by a stipe that is never concolorous with the pileus, a mild (never acrid) taste, and spiral to parallel spore-ornamentation.

Including *Lactarius vesterholtii*, *Lactarius* subg. *Plinthogali* is now represented by 11 taxa from India. A key is provided below for their easy identification.

Key to the identification of taxa of *Lactarius* subg. *Plinthogali* in India

1. Spore-ornamentation <1.5 µm 2
1. Spore-ornamentation >1.5–2.7 µm. 6
2. Pleuromacrocystidia present 3
2. Pleuromacrocystidia absent 4
3. Latex does not fade to yellowish after turning carrot red; wide spore range (6.4–9.7 × 5.6–8 µm); basidia longer (40.3–68.4 × 7–16 µm) ..... *L. subvernalis* var. *himalayensis*
3. Latex fades to yellowish after turning carrot red; spore range narrow (6.9–7.7 × 5.4–7.2 µm); basidia shorter (37–48 × 8–11 µm) ..... *L. subvernalis* var. *albo-ochraceus*
4. Pileus small (< 30 mm); stipe narrow. ..... *L. crenulatus*
4. Pileus large (>30 mm); stipe wide 5
5. Pileus yellow-brown to whitish; spore print pinkish buff; context salmon when exposed ..... *L. fuliginosus*
5. Pileus dark grey-brown to blackish brown; spore print bright ocher; context pale reddish brown when exposed ..... *L. picinus*
6. Spore ornamentation never reticulate, of parallel wings >2.5 µm high... *L. montoyae*
6. Spore ornamentation a partial to incomplete reticulum, always <2.5 µm high 7
7. Latex white, unchanging and not staining lamellae ..... *L. lignyotellus*
7. Latex white, turning lamellae pinkish to yellowish pink or saffron 8
8. Lamellar edge emarginate, unchanging or becoming orange after bruising; pileopseudocystidia present or absent; spore-ornamentation ≤2.3 µm high ... 9
8. Lamellar edge emarginate or marginate, but not with the above combination of characters 10
9. Lamellae distant, unchanging after bruising; context unchanging with guaiacol; spore print ochraceous; pileopseudocystidia present ..... *L. croceigalus*
9. Lamellae very crowded, becoming orange after bruising; context reddish brown with guaiacol; spore print light yellow; pileopseudocystidia absent ..... *L. vesterholtii*
10. Lamellae emarginate, lamellar edge concolorous with the surface; stipe stuffed; found under *Quercus*, *Rhododendron* association ..... *L. lignyotus* var. *lignyotus*
10. Lamellae marginate, lamellar edge brownish black; stipe always hollow; found under *Abies*, *Picea* association ..... *L. lignyotus* var. *canadensis*

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The authors are grateful to Dr. P. Singh, the Director, Botanical Survey of India (BSI), Kolkata (India) and Department of Forest, Environment and Wild Life Management, Government of Sikkim for providing all kinds of facilities during this study. They are thankful to Dr. N.S. Atri and Dr. K.B. Vrinda for kindly reviewing the manuscript and providing valuable suggestions. Thanks are also due to Dr. Md. N. Aziz (BSI, Cryptogamic Unit, Howrah), Dr. B.S. Kholia (BSI, SHRC, Gangtok) for helping the senior author in many ways. Assistance during macrofungal exploration to the North district of Sikkim rendered by A. Parihar (BSI, Cryptogamic Unit, Howrah) and S. Pradhan (BSI, SHRC, Gangtok) is also duly acknowledged.

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MYCOTAXON

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Volume 129(2), pp. 485 *October–December 2014*

Regional annotated mycobiotas new to the Mycotaxon website

Abstract — Mycotaxon is pleased to announce the posting of a new species distribution list by Barbosa, Machiner, Barbosa and Gusmão, who review the fungi from Serra da Jibóia, Brazil. This brings to 113 the number of free access mycobiotas now available on the Mycotaxon website: http://www.mycotaxon.com/resources/weblists.html

South America

Brazil

Flávia R. Barbosa, Monique Machiner, Gleyson Cristiano K. Barbosa, & Luís F. P. Gusmão. A checklist of the fungi recorded from Serra da Jibóia, Bahia state, Brazil. 33 p.

Abstract—Serra da Jibóia is one of the priority areas for the conservation of biodiversity located in the semiarid Caatinga biome of Northeast Brazil. Two hundred and fifty-nine species of fungi (mitosporic and meiosporic ascomycetes and basidiomycetes) were reported from Serra da Jibóia and are presented here. These species are distributed in 155 genera among which *Dictyochaeta* was the most highly represented genus with nine species. Inventory of the Serra da Jibóia region has thus far contributed to the description of one new genus and seven new species to science; six species are considered rare while 121 are new records of fungi. These data were based on literature records.

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Book reviews and notices

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Mushrooms of the Midwest. By M. Kuo & A.S. Methven. 2014. University of Illinois Press, 1325 South Oak Street, Champaign IL 61820-6903. <www.press.uillinois.edu>. ISBN 978-0-252-07976-4, 440 p., 833 color photos, paper. Price $39.95.

Slightly over a century ago, North American mushroomers wishing to identify a mushroom from their local field or forest had their work cut out for them. Throughout the nineteenth century, identifications depended on local experts, oft-inaccessible scientific papers (Peck's New York botanical reports spring to mind), or sparingly illustrated British or Scandinavian tomes. Species concepts were almost invariably Europe based. The continent was too vast and mycologists too few to provide anything other than a general nudge in the right direction, so that the first order of business was usually amassing a sizable library before coming anywhere near the 'right' name. 'Iron Guts' McIlvaine (1902), motivated by an earnest desire to share the edible (and warn against the not-so-edible) fungal bounty, was arguably the first to publish a volume devoted solely to North American mushroom identification. Despite its decided eastern bias (and a disconcerting tendency to refer to plants and stems), it did offer the American public a means to key out mushrooms anywhere on the continent.

With the introduction of his 1918 The *Agaricaceae* of Michigan, Kauffman presented one of the first regional mushroom guides. What began as a 'series of surveys initiated in the summer of 1906' developed into a 'manual of considerable size' accessible to serious scientists. Kauffman's formidable and prolific student, A.H. Smith, refined that Michigan agaric manual into shorter and more accessible guides for the entire continent. Since Smith, the continent has welcomed a virtual cornucopia of general field guides, but mushroomers still need a more regional focus to identify their endemic and uncommon mushrooms with any accuracy.

Kauffman and Smith aside, there have been relatively few guides devoted to the central U.S.A. There are a fair number devoted to edible fungi—witness the lively and colorful books by Kuo (2007) and McFarland & Mueller (2009). But my library contains only three more comprehensive guides: Stubbs (1971), covering ~60 central Midwest species; the excellent Horn & al. (1993) keying out 150 Kansas Mushrooms; and the excellent 1989 guide to mushrooms of the midcontinental United States by Huffman & al. (see the review of the expanded 2008 revised edition, MYCOTAXON 112: 498–499).

This new guide to 557 taxa, therefore, is both overdue and welcome. MUSHROOMS OF THE MIDWEST covers 12 states extending "from the cold conifer bogs of northern Michigan to the steamy oak forests of Missouri" or from Ohio in the east to Kansas, Nebraska and the Dakotas in the west. Its authors are well known to legions of mushroom aficionados – Michael Kuo as principal developer of MushroomExpert.com and enthusiastic researcher of all things morel and Andy Methven, Eastern Illinois University professor, *Clavariadelphus* and *Lactarius* expert, and welcome North American foray instructor.

At 8×10″ and 440 pages, MUSHROOMS is not a pocket field guide. However the stiff paper cover reinforced at the spine and semi-gloss paper confer a hardiness appropriate for foray ground and picnic table. This durability does present some drawbacks: the color photos are neither as sharp nor as brilliant as those printed on glossy paper (or viewed on computer screen). The book is divided into 7 parts: introduction, collection and preservation techniques, microscope use, keys, mushroom descriptions, the phylogenetic scheme followed, and a combination glossary and index (with the taxa indexed both by genus and species).

The first three pages outline the effort needed to determine species, in particular noting the hours it took Smith and others to collect and describe them, the many contributions by 'citizen' mycologists, and the strides made through DNA sequence analyses. The next 8 pages briefly outline the equipment and procedures for mushroom identification and study followed by a 6-page guide on using a microscope.

There follow 58 pages of keys: a key to the major groups is followed by keys to the pink-spored gills; pale-spored gills 1&2; *Amanita, Lactarius, Russula,* dark-spored gills 1&2; terrestrial pored; lignicolous pored; chanterelles & trumpets; toothed fungi; puffballs & earthstars; cup mushrooms; morels, false morels, saddles; clubs & corals; and finally 'miscellaneous fungi' [e.g., *Hypomyces,* birds nests, stink horns, witch's butters].

Pages 80–396 cover the fungal taxa. Scientific names stand in easy-to-read upper case black font with the authorities fully spelled out in red upper case.

At least one colour photo—usually two or three—illustrates each species. Entry subheadings include ecology, fruitbody description, spore print color, chemical reactions, microscopic features (always spores, but often other diagnostic features such as pileipellis, cystidia, clamp connections, paraphyses, etc.), and comments.

At first riffle, I was perplexed by what seemed to be a disorganized hodge-podge—ascomycetes inter-mixed with basidios, gilled with pored, conks with puffballs—that obscured the basic organizational scheme: the alphabet. Kuo & Methven, however, explain their rationale:

> We chose this arrangement because contemporary studies have been shifting long-held assumptions about relationships between mushrooms (for example, the polypore *Bondarzewia berkeleyi* ... is more closely related to *Russula ballouii* ... than it is to most other polypores), so that a strictly taxonomic arrangement (phylum, class, order, family, genus, species) would not only seem odd to many readers but also probably be subject to change before the book was actually printed... Contemporary studies also make a traditional guidebook arrangement based on physical features like spore print color, the presence of gills or pores, and so on seem antiquated.

Once this pragmatic decision is accepted, there really is little difficulty in finding a species using the key. Such an arrangement, which is a strong incentive to USE the key, will no doubt frustrate the key-by-photo crowd but is more likely to produce an accurate identification.

The authors do not mention whether the photographs are vouchered by dried specimens, which we hope is the case. I have not taken the time to scrutinize each photo and description, but the photos appear to be accurately identified, although in one or two instances, I wondered why a newer name has not been used [not a *Lactifluus* to be found]! Nonetheless the nomenclature is more up-to-date than in most field guides, particularly given that we must adapt to an exponentially increasing number of name changes. The authors, who follow the taxonomic classification of Kirk & al. (2008), explain their (and our) quandary satisfactorily in their concluding chapter, "The evolutionary picture:"

> By the time the manuscript we're writing right now is sent to the printer, some of the information included in the tables below will have changed—and by the time you are reading this, even more changes may have occurred. The DNA revolution in mycology has only just begun...The tables below include only the genera represented in this book...

Overall, this is an excellent addition to the field guide pantheon, and a very welcome addition to my library.

Horn B, Kay R, Abel D. 1993. A guide to Kansas mushrooms. University Press of Kansas, Lawrence. 297 p., 150 spp.

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McIlvaine C, Macadam RK. 1902. One thousand American fungi (rev.). [1973 reissued: Something Else Press, Inc.], West Glover VT. 729 p., 182 pl., ~1000 spp.

Stubbs AH. 1971. Wild mushrooms of the central Midwest. 135 p., ~60 plates

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A field guide to Tasmanian fungi. By G. Gates & D. Ratkowsky. 2014. Tasmanian Field Naturalists Club, P.O. Box 68, Hobart, Tasmania 7001, Australia <http://tasfieldnats.weebly.com/bookshop.html>. ISBN 978-0-9578529-2-1, 254 p., colour photos (603 spp.), paper. Price AU$39.95.

Last week's post brought an unexpected jewel to our mailbox. Having the good fortune of being able to visit Australia twice, I have over the years added four beautifully illustrated guides to the venerable Cleland (1934–35) and scholarly four-volume FUNGI OF AUSTRALIA (1996–2003), including Fuhrer & Robinson (1992), Grey & Grey (1995), Bougher & Syme (1998), and Fuhrer (2005). One glance at this new field guide to 603 Tasmanian fungi, however, tells me my next sojourn down-under will be to that fungal paradise south of the mainland.

The authors bring considerable experience to their task, over the past 15 years having made more than 1000 forays collecting and documenting fungi in Tasmania. Genevieve Gates received her doctorate for her study of macrofungi on wood, litter, and soil in a Tasmanian eucalypt forest and is coauthor with Noordeloos of the *ENTOLOMATACEAE* OF TASMANIA (2012); when not foraying for mushrooms, Dr. David Ratkowsky serves as a statistician with the Tasmanian Institute of Agriculture (University of Tasmania).

The book is compact ('A5' or 6×8¼″) and designed for field use. The paper cover is durable and the semi-gloss papers should withstand moisture yet are smooth enough so that every photo is crisp, clear, and brilliantly colored. From the magnificent front cover image of *Aseroë rubra* to 'The Thumbs' on the back, the photos are stunning, virtually all with a focus razor-sharp enough to catch the smallest detail. One minor carp is that the narrow inside margins make it

necessary to hold the book open with both hands to read all of the text, with some left-hand index letters obscured.

The essential introductory section precedes keys to the gilled genera followed by a table of key features (spore print, substrate, stipe, comments) of the gilled genera. Species descriptions include gilled basidiomycetes (alphabetical by genus) followed by non-gilled basidiomycetes in artificial groupings—bird's nests, boletes, chanterelles, coral fungi & clubs, earthballs, earthstars, jelly fungi, leather fungi, polypores, puffballs, resupinates, spine fungi, stinkhorns—and concluding with ascomycetes (also alphabetized by genus). The volume ends with a bibliography, a glossary, the index, and five pages of Tasmania spectacular enough to entice anyone to the island, although the guide will definitely be useful on the main continent to the north.

The introduction covers the different biological strategies—ectomycorrhizal, saprotrophic, pathogenic, parasitic, coprophilous, symbiotic (lichens)—and notes the important diagnostic macrofeatures used in the book—substrate, spore print colour, veils, gills & gill-like structures [with 3 ½ pages of photos of gill types], color changes with bruising, odours. With respect to nomenclature, the authors explain:

> We have used ... Index Fungorum as our source of the most up-to-date names for genera and species. Sometimes we did not adopt the proposed name but used an older synonym because that is the more familiar name or fitted better in the context of the book. The alternative name is given in brackets. For most unknown species we have used a 'tag name' and this is in inverted commas.... The abbreviation 'aff.' (*affinis* – similar to) means that we are not absolutely sure it is this species but it has similar characters, 'cf.' (compare with) is a stronger similarity, 'ined.' means it is in the process of being published and 'nom. prov.' (provisional name) means that it should be given that name when it is published. The rapid rise of molecular work is resulting in name changes of fungi. This is not a problem as these changes can be tracked using Index Fungorum, which gives the current name and synonyms and also the article in which the change of name is proposed.

The description pages each offer 2–3 species with generic names at top. At least one photo (often with detail insets) illustrates each species, accompanied by the scientific name, a short description with comments, and a fruiting season diagram. Descriptions are very brief, but the photos (taken in the natural habitat) are sufficiently clear to permit easy identification. Of particular note is the unusually large and welcome number of *Entoloma* species (16+ pages), a genus usually given short shrift in general field guides. It helps to have an expert author at hand.

This is a honey of a field guide, which I hope to take along the next time I have an opportunity to fly to Tasmania. Buy two, though, if for no other reason than to keep one set of pictures pristine.

Bougher NL, Syme K. 1998. Fungi of Southern Australia. University of Western Australia Press, Perth. 391 p., watercolor drawings, 125 spp.

Cleland JB. 1934–1935. Toadstools and mushrooms and other larger fungi of South Australia, Parts I and II. [Photolitho reprint 1976, A.B. James, Government Printer, South Australia]. 362 p., 77 b&w figs.

Fuhrer B. 2005. A field guide to Australian fungi. Bloomings Books Pty Ltd, Melbourne. 360 p., 548 colour pl., >500 spp.

Fuhrer B, Robinson R. 1992. Rainforest fungi of Tasmania and south-east Australia. CSIRO, East Melbourne. 95 p., ~109 pl.

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Grey P, Grey E. 2005. Fungi down under—the Fungimap guide to Australian Fungi. Fungimap, Royal Botanic Gardens Melbourne. 146 p., over 200 pl., ~150 spp., distribution maps, colour chart.

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BOOK ANNOUNCEMENT

California mushrooms. The comprehensive identification guide. By D.E. Desjardin, M.G. Wood & F.A. Stevens, 2014. Timber Press Inc., 133 S.W. Second Avenue, Suite 450, Portland OR 97204-3527, <timberpress.com>. ISBN 978-1-60469-353-9. 560 p., over 700 colour photos. Price $60

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Nomenclatural novelties and typifications proposed in Mycotaxon 129(2)

Coccomyces alienus Y.R. Lin & Xiao Y. Wang, p. 298
Cytospora rostrata C.M. Tian & X.L. Fan, p. 307
Dictydiaethalium dictyosporangium B. Zhang & Yu Li, p. 456
Diploschistes xinjiangensis A. Abbas & S.Y. Guo, p. 469
Entoloma discoloratum Largent, p. 343
Entoloma guttulatum Largent, p. 354
Entoloma hymenidermum Largent, p. 334
Entoloma kewarra Largent, p. 345
Entoloma pamelae Largent, p. 348
Entoloma rugosiviscosum Largent, p. 351
Entoloma violaceotinctum Largent, p. 339
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